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VOLUME IV

DECEMBER, 1945

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STUDIES

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INSTITUTUM DIVI THOMAE

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Volume IV

FOREWORD

With this issue, publication of the *Studies of the Institutum Divi Thomae*, interrupted by the war, is resumed. Because of the interval since the appearance of the last number (Vol. 3, No. 1, Nov., 1941) it has been decided to allow that number to constitute the whole of Volume 3. Accordingly, index and title page for binding of Volume 3 are included with the present number.

In the future, one volume of at least one number will be issued per year. The present number (Vol. 4, Dec., 1945) comprises the whole of Volume 4 and contains index and title page.

As has been customary, the majority of the papers from the Institutum Divi Thomae will continue to be published in outside journals. It is expected that the contents of the *Studies* will be representative of the researches being conducted in the Institutum laboratories. The avenue of publication, however, remains at the discretion of the individual author. Appended to this issue is a list of papers published in outside journals since the appearance of the last number of the *Studies*.

STUDIES
OF THE
INSTITUTUM DIVI THOMAE

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Address communications to the Chairman

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Nihil Obstat William J. Gauche, S.T.D.
Imprimatur John T. McNicholas, Archbishop of Cincinnati
The Nihil Obstat and Imprimatur neither favor nor oppose opinions or theories here recorded. They are declarations that there is nothing in the Studies of the Institutum Divi Thomae contrary to faith or morals.

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STUDIES OF THE
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VOLUME 4 DECEMBER, 1945

MODE OF ACTION OF YEAST EXTRACTS IN ACCELERATING PEROXIDASE ACTIVITY

CORNELIUS W. KREKE AND
SISTER M. DOMITILLA BARTLETT, R.S.M.

In previous communications, yeast extracts have been shown to accelerate the activity of peroxidase[1] and catalase[2]. The acceleration appears to be concerned with the enzyme itself since poisoning by cyanide and azide could be overcome by the extract. Decomposition of hydrogen peroxide by hemin was not accelerated by the yeast extract[2]. The yeast extract *per se* has only a slight peroxidase and no catalase activity[1, 2].

For some time yeast extracts have been known to stimulate the respiration of various types of cells such as yeast, skin, and liver[3]. This stimulating activity of the extract apparently is concerned with the cytochrome portion of the respiratory chain of enzymes[2] because cyanide and azide poisoning of this portion of the enzyme chain could be overcome by the extract while amyl alcohol and urethane poisoning of the dehydrogenating portion of the chain could not be overcome[2, 4].

The stimulation of respiration is probably a by-passing of the iron oxidation enzymes by the extract which carries

[1]KREKE, C. W., BARTLETT, SR. M. D., AND SUTER, SR. M. ST. A.: *Nature* **154** (1944): 268.

[2]KREKE, C. W., BARTLETT, SR. M. D., AND SMALT, SR. M. A.: *J. Biol. Chem.* **158** (1945): 469.

[3]COOK, E. S., KREKE, C. W., AND NUTINI, L. G.: *These Studies* **2** (1938): 23.

[4]KREKE, C. W., AND SUTER, SR. M. ST. A.: *J. Biol. Chem.* **160** (1945): 105; KREKE, C. W., AND SUTER, SR. M. ST. A.: *These Studies* **4** (1945): 85.

oxygen[2]. The acceleration of peroxidase and catalase, however, is a direct action on the enzyme itself since, as stated previously the extract *per se* has no significant peroxidase or catalase activity.

The present paper is a report of further studies on the mechanism of acceleration by yeast extract of an enzyme system containing peroxidase. The technique employed is based on the conversion of pyrogallol to purpurogallin by peroxidase under a series of experimental conditions designed to determine the specific rôle of the yeast extract in the increased production of purpurogallin.

1. Yeast extract was added to a system containing pyrogallol, hydrogen peroxide, and peroxidase and the yield of purpurogallin was compared with that in a control system.

2. A test was made to determine if there were ether-soluble coloring substances in the yeast extract which could be responsible for the increased amount of purpurogallin.

3. The substrate pyrogallol was omitted to determine if yeast extract could act as a substrate in the control system.

4. Peroxidase was omitted to determine if the yeast extract could act as a catalyst oxidizing pyrogallol in the presence of hydrogen peroxide.

5. The enzyme was poisoned with cyanide and the extract was added to see if the effect of the poison could be overcome by the extract. The procedure was repeated using azide instead of potassium cyanide as a poison.

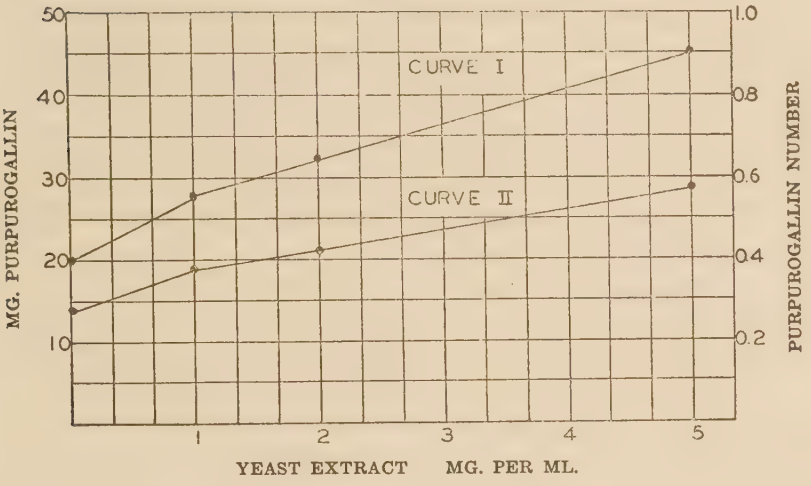
PROCEDURE

The method used in determining peroxidase activity was that of Willstätter modified by Bancroft and Elliott[5].

A peroxidase preparation was obtained by extracting 5 gm. of dried horse-radish root with 50 ml. of water at room tem-

[5] Willstätter, R., and Pollinger, A.: *Ann. Chem.* **430** (1923): 290.

FIG. 1. *Effect of Yeast Extract on the Production of Purpurogallin by Peroxidase.*



perature and filtering off the insoluble residue. Each ml. of the filtrate contained 37.7 mg. dry weight. Two ml. of the extract was diluted to 125 ml. with redistilled water containing 0.313 gm. of pure pyrogallol, and 12.4 ml. of M/300 copper-free potassium dihydrogen phosphate adjusted to pH 6 with a few drops of normal, pure sodium hydroxide solution. The mixture was placed in a liter Erlenmeyer flask in a water bath at 20° C. and vigorously stirred with a mechanical stirrer throughout the experimental period. When the solution reached a temperature of 20°, zero time, 0.625 ml. of 0.5 per cent hydrogen peroxide was added, and at the same time, from a separatory funnel with a stopcock adjusted to deliver 50 ml. per minute, 250 ml. of a solution was added containing 25 ml. of buffer, pH 6, 0.625 gm. pyrogallol and 160 ml. of water. At the end of 5 minutes, 17.5 ml. of 44 per cent sulfosalicylic acid was added to stop the reaction and precipitate the proteins. The solution was filtered through No. 40 Whatman paper and extracted three times with 33 ml. portions of C.P. anhydrous

TABLE I. *Effect of Peroxidase on Yeast Extract*

Flask*	Pyrogallol	Yeast Extract	Total Purpurogallin	Purpurogallin Number
	gm.	*gm.*	*mg.*	
I. Control.....	0.9375	....	21.2	0.28
II. Peroxidase +Yeast Ext. $+H_2O_2$.....	.....	1.50	0.0	0.00
III. Control +Yeast Ext $+H_2O_2$.....	0.9375	1.50	32.8	0.43

*Each flask contained 75.4 mg. of dry peroxidase extract.

ether to remove the ether-soluble purpurogallin. The purpurogallin yield was determined with a photodensitometer and is reported as the total number of mg. produced. These values were converted into purpurogallin numbers (P.N.) which are included in the tables.

In the system as described herein, the yield of purpurogallin was between 15 and 25 mg. as was recommended by Willstatter[5]. Preliminary tests had showed that in the range of 0.1 to 2 ml. of peroxidase preparations, the amount of purpurogallin was a straight line function. The P.N. in this range remained practically constant.

EXPERIMENTAL

1. *Effect of Yeast.* Various amounts of a sterile yeast extract, prepared as previously described[3] and possessing the ability to increase respiration in yeast and animal tissue, were added to the pyrogallol-peroxidase solution immediately before the beginning of the five-minute experimental period. The amounts of yeast extract used were sufficient to make concentrations of 1, 2, and 5 mg. per ml. in the solution.

In Fig. 1 it is seen that with increasing concentrations of the yeast extract there was an increase both in the total number of mg. of purpurogallin (125%) and in the purpurogallin number (128%). This is one of five experiments which showed the same results. In the remaining experiments of the series the final concentration of yeast extract in the system was 5 mg.

2. *Yeast Extract As a Source of Color.* When pyrogallol was omitted from the system, the ether extract was colorless, showing that the yeast extract itself neither contained nor produced ether-soluble coloring material (Table I), and so it had not contributed to the color reading of the increased purpurogallin yield.

3. *Yeast Extract As a Possible Substrate.* To determine if yeast extract acted as a substrate, pyrogallol was omitted from the system, as described in Part 2. No ether-soluble coloring materials were found, showing that under the conditions of the reaction, the yeast extract did not contain material that acted as a substrate for peroxidase.

4. *Yeast Extract As a Possible Catalyst.* When peroxidase was omitted from the control system, there was a small amount

TABLE II. *Peroxidase Action of Yeast Extract*

Flask	Pyrogallol	Dry Peroxidase Extract	Yeast Extract	Total Purpurogallin	Purpurogallin Number
	gm.	*mg.*	*gm.*	*mg.*	
I. Control	0.9375	75.4	....	21.2	0.28
II. Yeast Ext.				3.5	
+Pyrogallol				3.5	
$+H_2O_2$	0.9375	....	1.50	3.6	
III. Yeast Ext.					
$+H_2O_2$	.....	....	1.50	0.0	
IV. Pyrogallol				1.2	
$+H_2O_2$	0.9375	....	....	1.3	

TABLE III. *Antagonism of Action of Yeast Extract and Potassium Cyanide on Peroxidase*

Flask*	Yeast Extract	0.75 M KCN	Total Purpurogallin	Purpurogallin Number
	gm.	*ml.*	*mg.*	
I. Control.....	....	..	22.0	0.29
II. Control +Yeast Ext....	1.50	..	32.8	0.43
III. Control +KCN.....	....	1	14.0	0.19
IV. Control +Yeast Ext. +KCN.....	1.50	1	20.0	0.27

*Each flask contained 0.9375 gm. of pyrogallol and 75.4 mg. of dry peroxidase extract.

of autoxidation of the pyrogallol by the hydrogen peroxide, 1.2 – 1.3 mg. of purpurogallin being produced. This autoxidation was increased with the addition of the yeast extract (5 mg./ml.) to a value of 3.5 – 3.6 mg. From the data obtained under these experimental conditions it appears that yeast extract had a definite, although slight, peroxidase-like activity, but this action fails to account for the purpurogallin increase of 8.5 mg. in the complete control system when 5 mg. of yeast extract was added (Table II).

5. *Poisoning of the Enzyme.* When 1 ml. of 0.75 M potassium cyanide was substituted for 1 ml. of water in the control system, the yield of purpurogallin was lowered from 22 to 14 mg. (Table III). This was increased to 20 mg. in the presence of yeast extract (5 mg./ml) showing that the extract overcame the cyanide depression of the peroxidase activity.

Azide poisoning of the enzyme action produced qualitatively similar results which have been published.[1]

DISCUSSION

From the experimental evidence it is apparent that yeast extract does not increase the production of purpurogallin by supplying ether-soluble coloring material, by acting as a substrate, or by acting as a catalyst.

Since the yeast extract could offset the poisoning of peroxidase by potassium cyanide and sodium azide, the action of the extract is probably directly concerned with the action of the peroxidase itself. The fact that the yeast extract accelerates catalase decomposition of hydrogen peroxide lends weight to this theory of the mode of action of the extract.

SUMMARY

1. Yeast extract accelerated the peroxidase-pyrogallol-hydrogen peroxide system.

2. It was shown experimentally that the yeast extract did not act as substrate nor enzyme and did not supply ether-soluble coloring material, but was concerned with the enzyme itself. The yeast extract was used to overcome cyanide and azide poisoning of the enzyme peroxidase.

Original Manuscript Submitted
July 3, 1943.

EFFECT OF RABBIT SPLEEN EXTRACT AND SPLEEN PULP ON MOUSE CARCINOMA[1]

SISTER CRESCENTIA GIERSCH, C.S.J.[2]

MUCH of the literature up to 1940 concerning attempts to induce a resistance against tumors in animals has been reviewed by Fardon, Brotzge, and Loeffler[3]. Gross[4] brought about regression of tumors in C_3H mice by intradermal immunization, and he suggested that the immunity is specific against the tumor used for inoculation. Lewis[5] stated that induced spindle cell mouse sarcomas brought about, in an alien strain host, an immunity from the growth of the autograft or another graft of the same tumor. Cheever[6], likewise, produced immunity in rabbits against the Brown-Pearce carcinoma by intratesticular and intradermal inoculations of testicular and metastatic tumor nodes. After regression of the growth the animals were refractory to subsequent implants of the same type of tumor. Fardon, Brotzge, and Loeffler[3] were able to induce immunity against mammary carcinoma No. 15091a in mice by using mouse spleen and mouse embryo extracts.

In this paper the results of three attempts to build up a resistance against carcinoma No. 15091a (Roscoe B. Jackson Memorial Laboratory) are reported. Jack-rabbit-spleen

---

[1]From the Marymount College Unit of the Institutum Divi Thomae, Salina, Kansas.

[2]Assistance in the experimental work was given by Sister Mary Grace Waring, C.S.J.

[3]FARDON, J. C., BROTZGE, G. C., AND LOEFFLER, SR. M. K.: *These Studies* **3** (1941): 69.

[4]GROSS, L.: *Cancer Research* **3** (1943): 326.

[5]LEWIS, M. R.: *Bull. Johns Hopkins Hosp.* **67** (1940): 325.

[6]CHEEVER, F. S., AND JANEWAY, C. A.: *Cancer Research* **1** (1941): 28.

extract and spleen pulp were the agents used in attempting to produce immunization.

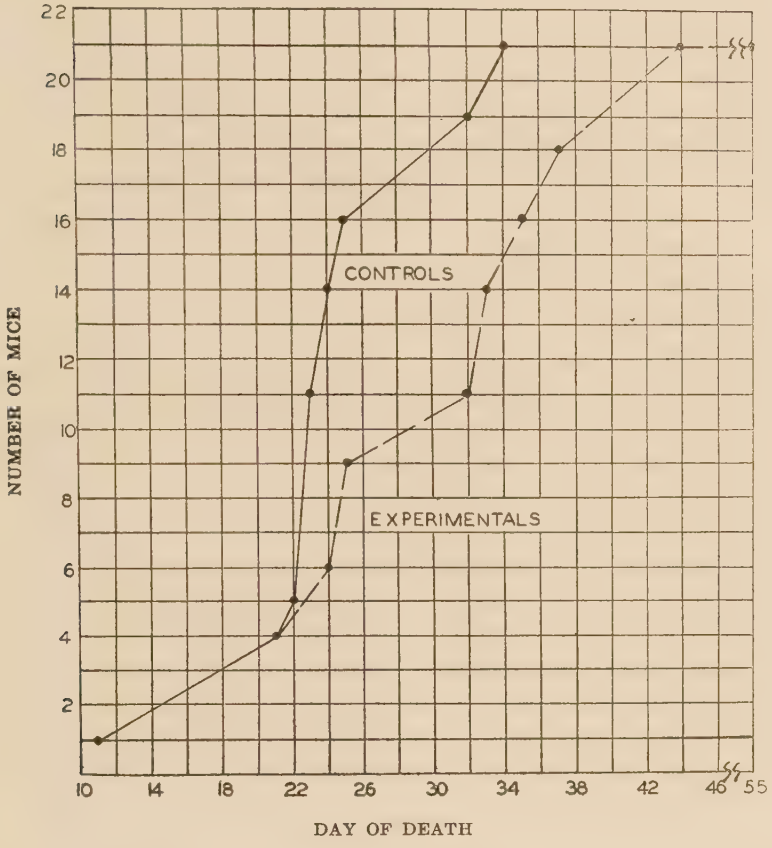
EXPERIMENTAL

One hundred and thirty-four of the ABC strain of mice of both sexes, two to three months old, and carcinoma No. 15091a were used throughout the experiment. One-half the animals served as the experimental, the other half as the control group.

Extracts. The spleens, removed under aseptic conditions from 15 jack rabbits, were placed in a slightly warmed sterile Tyrode's solution. After removing the connective tissue, the spleens were pulped through a fine wire mesh and 1 ml. of sterile Tyrode's solution was added for each 0.4 gm. of tissue weight. The suspension was mixed well and transferred to sterile centrifuge tubes in which it was frozen and thawed three times. Following this, the suspension was centrifuged for 20 minutes, and the supernatant fluid removed aseptically for inoculation. In every case the rabbit spleens were removed and the extract was prepared on the same day that the rabbits had been killed. One week after administration of the spleen extract, the experimental animals were inoculated with 0.25 ml. of the tumor suspension. The technique of Fardon, Brotzge, and Loeffler[3] was employed, except that Tyrode's solution was used as the suspending medium.

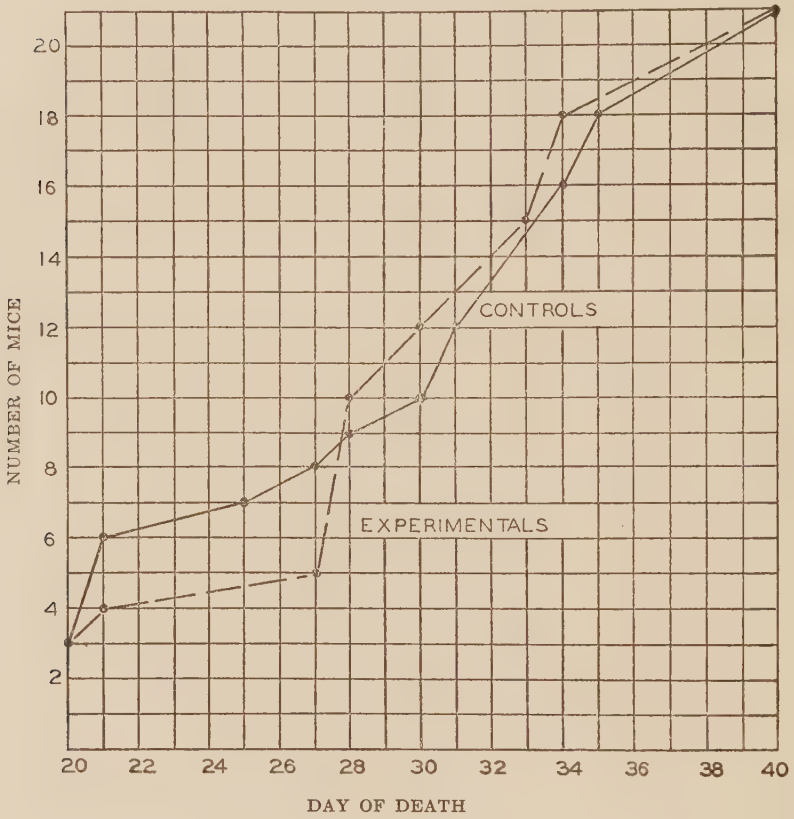
In the first trial, 25 mice were inoculated subcutaneously with 0.25 ml. of the spleen extract. At the same time each mouse in the control group of 25 animals received 0.25 ml. of the tumor suspension. Later, a second group of 21 mice was inoculated with extract prepared from 16 rabbit spleens. Each mouse received the usual dose of 0.25 ml. One week later, they were given a second inoculation of 0.25 ml. One week after the second treatment with spleen extract, the mice were each

FIG. 1. *Mortality among 21 mice inoculated with 0.25 ml. of rabbit spleen extract 14 and 7 days prior to receiving tumor transplants, the extract being prepared from pulp subjected to freezing and thawing.*



inoculated with 0.25 ml. of the tumor suspension, the 21 control mice each receiving 0.25 ml. of the tumor suspension. For the third group of mice the immunizing agent was not subjected to the freezing and thawing procedure. Each of the 21 mice was given two doses of 0.25 ml. of the pulp suspension at weekly intervals. Nine days later the experimental and the

FIG. 2. *Mortality among 21 mice inoculated with 0.25 ml. of rabbit spleen pulp 16 and 9 days prior to receiving tumor transplants. The spleen tissue was not subjected to a freezing and thawing procedure.*



control groups were inoculated with 0.25 ml. of the tumor suspension.

RESULTS AND CONCLUSION

In the first group of mice which received a single injection of spleen extract prior to inoculation with the tumor suspension,

the tumor was viable in 100 per cent of the experimental and control animals 9 days after inoculation. Two weeks later the tumors of both groups were measured, and the animals were killed. The average size of the tumor for the experimental group was 19.1 x 13.3 mm; that of the control group, 21.4 x 15.6 mm.

The second group of experimental animals which received two preliminary inoculations with rabbit spleen extract seemed to show a very slight degree of resistance to tumor development. The tumors were viable in but 90.4 per cent of these as compared with 100 per cent takes in the control animals. However, the most interesting results seemed to be the apparent effect which the extract had upon the longevity of the experimental animals in contrast to the controls (Fig. 1). After inoculation with the spleen extract the average length of life of the experimental animals was 31.3 days as compared to 24.7 days in the control animals. The percentage increase in life span was 20.8 which statistically is not significant.

Apparently jack-rabbit-spleen pulp as used in the third group of mice had no immunizing effect since tumors developed in 100 per cent of both experimental and control animals and there was no pronounced difference in the mortality rate of the two groups (Fig. 2). The average length of life after tumor inoculation in the experimental was 30.5 days as compared with 29.5 days for the control animals. This may possibly be due to the introduction of too much foreign protein which lowered the resistance of the animals, or to the fact that the immunizing factor was not released in the preparation of the pulp.

SUMMARY

1. Rabbit spleen extract prepared by alternate freezing and thawing may possibly have some effect upon the resistance of mice of the ABC strain against carcinoma No. 15091a.

2. Rabbit spleen pulp did not protect ABC mice against carcinoma No. 15091a.

Original Manuscript Submitted
August 16, 1943

EFFECT OF YEAST EXTRACT ON VARIOUS PEROXIDATIC REACTIONS

CORNELIUS W. KREKE AND
SISTER M. DOMITILLA BARTLETT, R.S.M.

In a preceding paper[1] an extract from yeast cells was used to accelerate the oxidation of pyrogallol by horse-radish root peroxidase. This phenomenon was shown to be concerned directly with the iron-porphyrin enzyme, since the yeast extract did not act as substrate or enzyme in the system, and since the cyanide and azide poisoning of the enzyme could be completely overcome by the extract.

The present paper is a report of experiments showing that the action of the extract is not specific in its acceleration of the oxidation of pyrogallol and that its action is not limited to the horse-radish peroxidase.

EXPERIMENTAL

A yeast extract, prepared as previously described[2], was tested for activity in the oxidation of benzidine, *p*-phenylenediamine, cresol mixture, and malachite green by *a*) horse-radish root peroxidase, prepared as previously described[1] and *b*) an enzyme preparation obtained from yeast by the following method: Six one-day-old cultures of *S.cerevisiae* on Sabouraud's medium were washed with physiological salt solution and ground in a mortar with clean sand. The sand and cellular debris were filtered off.

The benzidine solution was prepared by saturating 50 per cent alcohol. Of the substrates used in the testing, benzidine

---

[1]KREKE, C. W., AND BARTLETT, SR. M. D.: *These Studies* **4** (1945): 1.

[2]COOK, E. S., KREKE, C. W., AND NUTINI, L. G.: *These Studies* **2** (1938): 23.

TABLE I. *Optimum pH Values for Various Peroxidase Substrates*

pH	Benzidine	*p*-Phenylenediamine	Cresol Mixture	Malachite Green
4.68	+	+++	+++	+++
5.35	+	++	++	+++
5.65	++	++	++	+++
6.00	++	++	+	−
6.50	+++	+	+	−
7.30	+++	+	+	−

gave the most satisfactory results and these are reported in detail. Slight changes in acidity greatly affected the rate of oxidation, and the optimum pH varied with the substrate used (Table I). The buffer consisted of a mixture of sodium dihydrogen phosphate and disodium phosphate, the pH being determined with a glass electrode.

The procedure used was as follows: To the substrate in a test tube containing 4 drops of buffer, pH 7.3, was added the horse-radish extract or the yeast juice. At zero time, 2 drops of 3 per cent hydrogen peroxide was added and the color that developed in a standard period of time was compared with the color developed in a control tube in a similar period of time.

When horse-radish root peroxidase was used (Table II), yeast extract accelerated the oxidation of benzidine; potassium cyanide retarded the oxidation of benzidine, and when yeast extract was present together with the potassium cyanide, the effect of the cyanide was offset. Experiments No. 5 and 6 show that the yeast extract was not acted upon by the peroxidase, nor did yeast extract oxidize the benzidine in the absence of the peroxidase. These results are qualitatively the same as those reported for pyrogallol[1].

TABLE II. *Effect of Yeast Extract on the Oxidation of Benzidine by Horse-radish Root Peroxidase*

Experiment*	1 Control	2 Control + Yst. Ext.	3 Control +KCN	4 Control + KCN + Yst. Ext.	5 Yst. Ext. $+H_2O_2+$ Benzidine	6 Yst. Ext. $+H_2O_2+$ Peroxidase
Physiol. salt soln., drop....	3	2	1	..	3	3
Benzidine soln., drop.....	1	1	1	1	1	..
M/500 KCN, drop.....	..	..	2	2	..	..
Yeast Ext. 30 mg./ml., drop.	..	1	..	1	1	1
Peroxidase preparation, drop.....	1	1	1	1	..	1
Result: Brown color in a few seconds.....	+++	++++	+	+++	No color after 24 hr.	No color after 24 hr.

*Each tube initially contained 2 drops of buffer mixture, pH 7.3. At zero time 2 drops of 3% H_2O_2 was added to the mixture of buffer, substrate, yeast extract, and peroxidase.

When the enzyme preparation from yeast cells was substituted for the horse-radish root peroxidase (Table III), the results were qualitatively the same in all of the experiments. The yeast enzyme as prepared was not very active and a longer time was required for the development of color than was the case with peroxidase. It is apparent from the data that yeast extract, in the presence of the yeast enzyme, accelerated the enzyme and completely offset the cyanide depression of the enzyme preparation.

DISCUSSION

The experimental data show that yeast extract can accelerate the oxidation of the substrates benzidine, *p*-phenylenediamine,

TABLE III. *Effect of Yeast Extract on the Oxidation of Benzidine by Yeast Juice*

Experiment*	1 Control	2 Control + Yst. Ext.	3 Control + KCN	4 Control + KCN + Yst. Ext.	5 Yst. Ext. + H_2O_2 + Benzidine	6 Yst. Ext. + Yeast Juice + H_2O_2
Physiol. salt soln., drop....	1	..	..	..	1	1
Benzidine soln., drop.....	1	1	1	1	1	..
M/500 KCN, drop.....	..	..	1	1	..	..
Yeast ext. 10 mg./cc., drop.	..	1	..	1	1	1
Yeast juice (enzyme), drop	1	1	1	1	..	1
Results: Brown color in 4 min..	+	+	−	+	−	−

*Each tube initially contained 4 drops of buffer, pH 7.3., and 2 drops of 3% H_2O_2.

malachite green, and cresol and, therefore, the action of the yeast extract is not specific for pyrogallol oxidation. When these same substrates were oxidized by yeast juice the action was accelerated by the yeast extract. From this it is inferred that the action of yeast extract is not specific for horse-radish root peroxidase. Further experimental evidence is presented that the yeast extract does not act as substrate or enzyme since there was no oxidation in the absence of either substrate or peroxidase in this series of experiments.

Yeast extract has been found to stimulate the respiration of various cells, including yeast,[3] liver,[4, 5] and skin,[4, 5] and the

[3] KREKE, C. W., AND SUTER, SR. M. ST. A.: *J. Biol. Chem.* **160** (1945): 105.

[4] KREKE, C. W., BARTLETT, SR. M. D., AND SUTER, SR. M. ST. A.: *Nature* **154** (1944): 268.

[5] KREKE, C. W., AND SUTER, SR. M. ST. A.: *These Studies* **4** (1945): 85.

action seemed to be concerned with the cytochrome portion of the respiratory chain since the extract overcame respiratory depression by the iron poisons, cyanide and azide. Yeast extracts also accelerate the decomposition of hydrogen peroxide by catalase[4, 6].

It is interesting to note in the 3 types of acceleration that the enzymes influenced are iron-porphyrin compounds.

SUMMARY

Yeast extracts accelerated the oxidation of several amino and hydroxy compounds by horse-radish root peroxidase in the presence of hydrogen peroxide; the extracts offset the depression of enzyme activity by potassium cyanide. Similar results were obtained when an enzyme preparation from yeast cells was substituted for the horse-radish root peroxidase.

Original Manuscript Submitted
September 17, 1943

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[6] KREKE, C. W., BARTLETT, SR. M. D., AND SMALT, SR. M. A.: *J. Biol. Chem.* **158** (1945): 469.

ULTRAVIOLET ABSORPTION OF SOME ORGANIC COMPOUNDS[1]

Part II. Aromatic Compounds[2]

SISTER MIRIAM MICHAEL STIMSON, O.P., AND
SISTER MARY AGNITA REUTER, O.P.[3]

A detailed review of the ultraviolet absorption spectra of the aromatic hydrocarbons was recently published by Jones[4] in which the influence of structural factors on spectra is considered for about 370 hydrocarbons. These include various alkyl, alicyclic, alkene, and cyclo-alkane derivatives. For the present purposes, therefore, it suffices to present the range of absorption for the hydrocarbon ring systems in a diagram modified after Conrad-Billroth[5] (Fig. 1). It is evident from this scheme that the differences in the spectra for the various condensed ring systems are sufficient to permit identification of a system from the general absorption curve. Derivatives of the simplest aromatic hydrocarbon, benzene, will be considered in more detail because they constitute the most widely used aromatic compounds.

Hydrocarbons

The absorption of benzene is shifted toward the visible by substitution of the hydrogen atoms and the shift depends on the nature, number, and position of the substituents[6]. The effect of the relatively inactive methyl group, when substituted

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[1]This review covers data reported since 1920.

[2]Part I, Aliphatic Compounds: *These Studies* **3** (1941): 167.

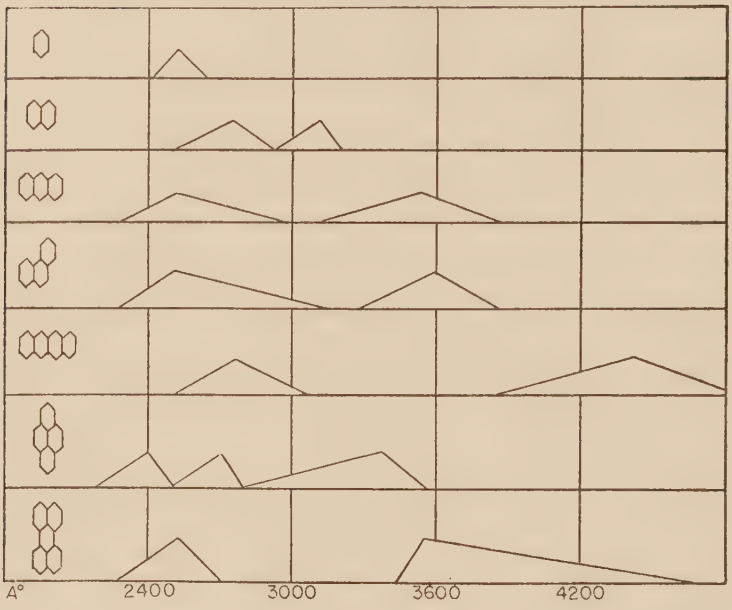
[3]From the Siena Heights College Unit of the Institutum Divi Thomae, Adrian, Michigan.

[4]Jones, R. N.: *Chem. Rev.* **32** (1943): 1.

[5]Conrad-Billroth, H.: *Z.physik. Chem.* **B 15** (1931): 1.

[6]Idem: *ibid.* **B 20** (1933): 227.

FIG. 1. *Range of ultraviolet absorption of hydrocarbon ring systems* (Conrad-Billroth[5]).



for successive hydrogen atoms, is shown in Table I adapted from Conrad-Billroth[7] and from Hillmer and Schorning[8]. From these data it is seen that substitution of the *para* position gives absorption of about twice the strength of that shown when the *ortho* or the *meta* positions are similarly filled. Billroth[7, 9] records a very pronounced fine structure in the spectrum of *m*-xylene and especially in that of *p*-xylene which is entirely absent in the completely methylated product. Steurer[10] does not completely agree with Billroth. Both workers are in accord, nevertheless, in that the appearance of of subsidiary peaks decreases with increased methylation.

[7]IDEM: *ibid.* **B 29** (1935): 170.

[8]HILLMER, A., AND SCHORNING, P.: *ibid.* **A 168** (1934): 81; PESTEMER, M., AND GÜBITZ, O.: *Monatsh. Chem.* **64** (1934): 426.

[9]CONRAD-BILLROTH, H.: *Z.physik. Chem.* **B 31** (1936): 475.

[10]STEURER, E.: *ibid.* **B 30** (1935): 157.

TABLE I. *Ultraviolet Absorption of Methyl Benzenes**

Name of Compound		Wavelength and Extinction of Maxima						
Benzene	Å	2385	2435	2490	2550	2610	2690	
	ε	60	112	204	234	186	10	
Toluene	Å	2440	2490	2545	2595	2620	2640	2685
	ε	90	176	235	266	305	200	270
Xylene 1, 2—	Å	2640	2710					
	ε	415	390					
1, 3—	Å	2585	2620	2645	2675	2725		
	ε	330	363	400	356	397		
1, 4—	Å	2515	2555	2595	2650	2675	2740	
	ε	330	400	545	780	726	960	
Trimethyl benzene								
1, 2, 3—	Å	2580	2625	2660	2700			
	ε	323	362	335	333			
1, 3, 5—	Å	2500	2585	2625	2650	2675	2715	
	ε	233	273	260	303	250	266	
1, 2, 4—	Å	2630	2680	2760				
	ε	600	735	835				
Tetramethyl benzene								
1, 2, 3, 4—		....	....	....	....			
1, 2, 3, 5—	Å	2680	2725	2775				
	ε	422	360	340				
1, 2, 4, 5—	Å	2690	2730	2780				
	ε	765	685	820				
Pentamethyl benzene	Å	2690	2755	2795				
	ε	367	308	293				
Hexamethyl benzene	Å	2705	2790					
	ε	330	240					

*Adapted from Conrad-Billroth[7] and Hillmer and Schorning[8].

Within any degree of methylation the order of increase in fine structure is:

Dimethyl benzene.....ortho- <meta- <para-
Trimethyl benzene.....1,2,3- <1,2,4- <1,3,5-
Tetramethyl benzene.....1,2,4,5- <1,2,3,5-

The effect of the chain itself was investigated by Hillmer and Schorning[11] who reported:

$C_6H_5CH_2CH_2CH_3$	Å		2608	2687		
	ϵ		214	209		
$C_6H_5CH_2CH=CH_2$ . . .	Å	2555	2605	2690		
	ϵ	209	219	182		
$C_6H_5CH=CHCH_3$	Å		2605		2865	3100
	ϵ		2089		447	141

The introduction of the allyl group as side chain on the benzene nucleus acts similarly to the *n*-propyl group in effecting a certain fusion of the secondary bands and a general shift toward the visible part of the spectrum. The extinction is increased over that of benzene. The propenyl side chain, however, completely changes the spectrum of the aromatic nucleus. This follows logically from the reactivity of this arrangement as postulated by Fuson[12] in the principle of vinylogy.

The combination of several nuclei as in biphenyl or compounds in which the nuclei are separated by one or more carbon atoms, has been studied considerably. Biphenyl[13] has a strong absorption band at 2550Å. Separation of the phenyl groups by a carbon atom causes a shift to longer wavelengths and a sharp fall in the long wavelength extinction. Further separation by saturated carbon atoms results in very little recovery of absorption strength. Unsaturation of the aliphatic chain causes a marked increase in absorption strength as well as

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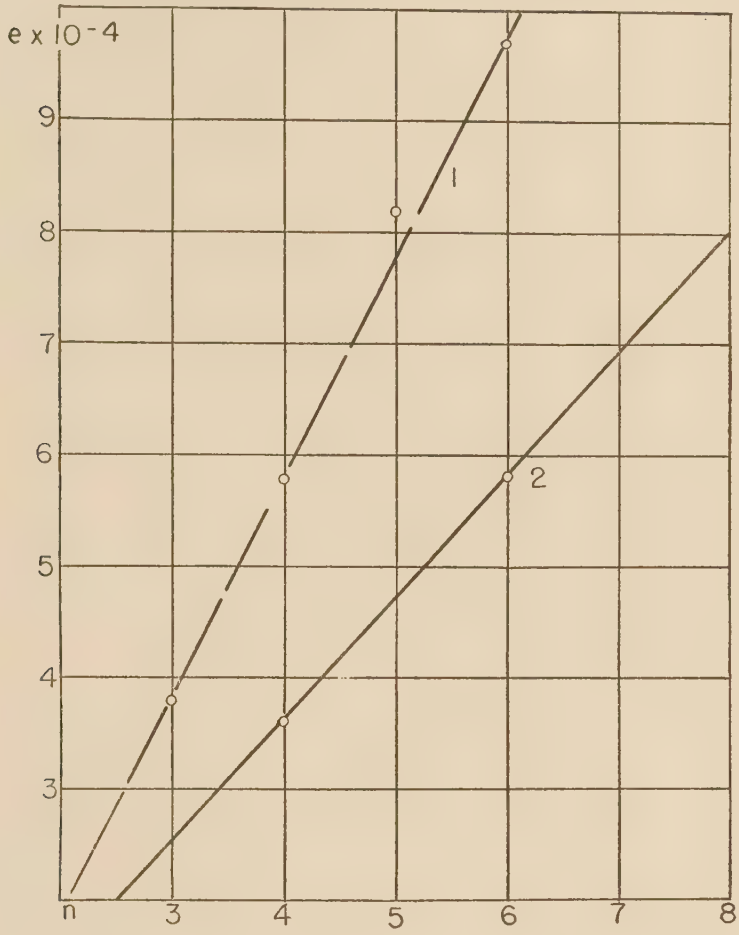
[11] Hillmer, A., and Schorning, P.: *ibid.* **A 167** (1933): 407; Allard, G.: *Helv. Chim. Acta* **19** (1936): 1270; Pestemer, M., and Wiligut, L.: *Monatsh. Chem.* **66** (1935): 119.

[12] Fuson, R. C.: *Chem. Rev.* **16** (1935): 1.

[13] Adam, T. C. C., and Russel, A.: *J. Chem. Soc.* (1930): 202; Craig, L. C., and Jacobs, W. A.: *J. Biol. Chem.* **139** (1941): 293; O'Shaughnessy, M. T., and Rodebush, W. H.: *J. Am. Chem. Soc.* **62** (1940): 2906.

FIG. 2. *Effect of unsaturation of aliphatic side chain on ultraviolet absorption of polyphenylpolyenes*[14]. Curve 1: diphenylpolyenes (conjugated double bonds). Curve 2: tetraphenylpolyenes (cumulative double bonds).



producing a bathochromic effect (Fig. 2)[14]. Hausser, Kuhn,

[14]RĂDULESCU, D., AND BARBULESCU, F.: *Ber. deut. chem. Ges.* **64** (1931): 2225; HAUSSER, K., KUHN, R., AND SMAKULA, A.: *Z. physik. Chem.* **B 29** (1935): 384; SMAKULA, A.: *ibid.* **B 25** (1934): 90; HAUSSER, K., KUHN, R., AND SEITZ, G.: *ibid.* **B 29** (1935): 391; KUHN, R., AND WALLENFELS, K.: *Ber. deut. chem. Ges.* **71** (1938): 783.

and Seitz extended in a quantitative way the observation of Kuhn and Winterstein[15] that in polyenes the color, in close approximation, can be considered an additive property of the number of double bonds. The linear relationship is brought out very well in Figure 27 of Branch and Calvin's work[16] in which the square of the wavelength of the first absorption band is plotted against the number of aliphatic double bands.

The necessity of coplanarity for resonance for stilbene is seen in the comparison of the spectra of the *cis* and the *trans* forms. *Trans*-stilbene is entirely coplanar and has an absorption band at longer wavelengths and greater strength than the *cis* form (Fig. 3) in which the coplanarity is reduced by steric hindrance. This effect is further illustrated by the work of Dimroth[17] in which spectra are reported for α-methyl and for α, β-dimethyl derivatives of *trans*-stilbene. The main absorption band of *trans*-stilbene is shifted 220Å to the violet accompanied by a molecular extinction drop of 5200 units on introduction of the first methyl group and shifts 310Å further on introduction of the second methyl with a fall in the extinction of another 6500 units. On this basis the data of Ley and Dirking[18] for the phenyl ethenes:

$C_6H_5-CH=CH-C_6H_5$	$(C_6H_5)_2C=CH-C_6H_5$	$(C_6H_5)_2C=C(C_6H_5)_2$
3000Å	3030Å	3090Å
log ϵ 4.34	log ϵ 4.23	log ϵ 4.17

may be interpreted to mean that the overall number of carbon atoms or the relative resolution of the rings coplanar to any one axis is greater when a phenyl group replaces a methyl

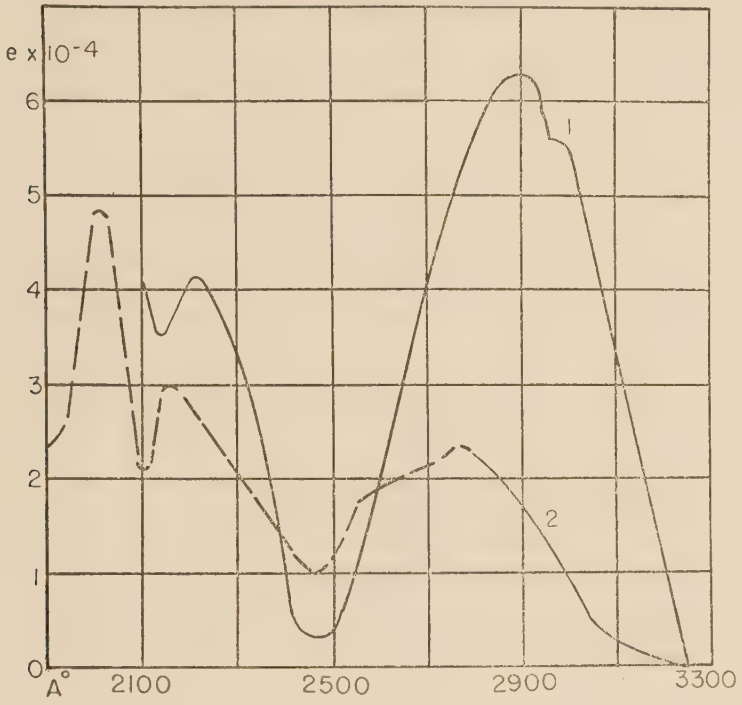
[15] Kuhn, R., and Winterstein, A.: *Helv. Chim. Acta* **12** (1929): 899.

[16] Branch, G., and Calvin, M.: The Theory of Organic Chemistry. Prentice-Hall, 1941, New York.

[17] Dimroth, K.: *Angew. Chem.* **52** (1939): 545; Arends, B.: *Ber. deut. chem. Ges.* **64** (1931): 1936.

[18] Ley, H., and Dirking, H.: *ibid.* **67** (1934): 1331.

FIG. 3. *Ultraviolet absorption spectra of trans- (Curve 1) and cis- (Curve 2) stilbene, after Smakula and Wasserman.*[18a]



group on the α carbon[19]. The study of the spectra of biphenyl along with those of naphthalene and fluorene led Carr, Pickett, and Voris[20] to conclude that from absorption data the formula proposed by Lothrop[21] for biphenylene is further substantiated.

Halogen Derivatives

The simple aromatic halides of the first four members of the halogen family have been studied in various positions. The

[18a] SMAKULA, A., AND WASSERMAN, A.: *Z. physik. Chem.* **A 155** (1931): 353

[19] STRAUS, F., KÜHNEL, R., AND HAENSEL, R.: *ibid.* **66** (1933): 1847.

[20] CARR, E. P., PICKETT, L. W., AND VORIS, D.: *J. Am. Chem. Soc.* **63** (1941): 3231.

[21] LOTHROP, W. C.: *ibid.* **63** (1941): 1187.

spectrum of fluorobenzene presented by Conrad-Billroth[22] shows only a slight depression and shift to shorter wavelengths when compared with that of benzene[23] and is essentially that of benzene. The spectra of chloro- and bromo-benzene also show considerable fine structure, which is less pronounced in the latter. This is not surprising if the halogen/hydrocarbon ratio be calculated. These ratios are approximately 0.25, 0.5, 1.0, and 1.5 for F, Cl, Br, and I, respectively. All characteristic aromatic absorption disappears in the spectrum of iodobenzene and the curve is of the type found for iodobutane or -heptane[24] and the spectrum probably represents mainly the halogen absorption. Figure 4, adapted from Conrad-Billroth,[22] shows the effect of substitution of fluorine in the *o*-, *m*-, and *p*-positions of toluene, respectively. The sharp fine structure of benzene is most pronounced in the last case, but with the exception of increase in the extinction there is relatively little difference in the three spectra. The diagrammatic representation of the data for the possible dichloro-, dibromo-, and diiodotoluenes in Figure 5 shows that the *para* substitution has a more marked bathochromic effect than either of the other two substitutions[25]. Conrad-Billroth[26] gives the following data for monochloro- and monobromo-xylenes:

	Halogen in the 1-position	
	Chloroxylenes	Bromoxylenes
1,2,4-.....	2705	2725
1,2,5-.....	2695	2710
1,2,6-.....	2660	2690
1,3,4-.....	2700	2700
1,3,5-.....	2675	2675

[22] Conrad-Billroth, H.: *Z. physik. Chem.* **B 25** (1934): 139.

[23] Mayneord, W. V., and Roe, E.: *Proc. Roy. Soc.* **A 152** (1935): 299.

[24] Stimson, Sr. M. M., and Reuter, Sr. M. A.: *These Studies* **3** (1941): 167, Fig. 1.

[25] Wolf, K. L., and Herold, W.: *Z. physik. Chem.* **B 13** (1931): 201.

[26] Conrad-Billroth, H.: *ibid.* **B 25** (1934): 217.

FIG. 4. *Effect of substituting fluorine in the ortho, meta and para positions of toluene*[22].

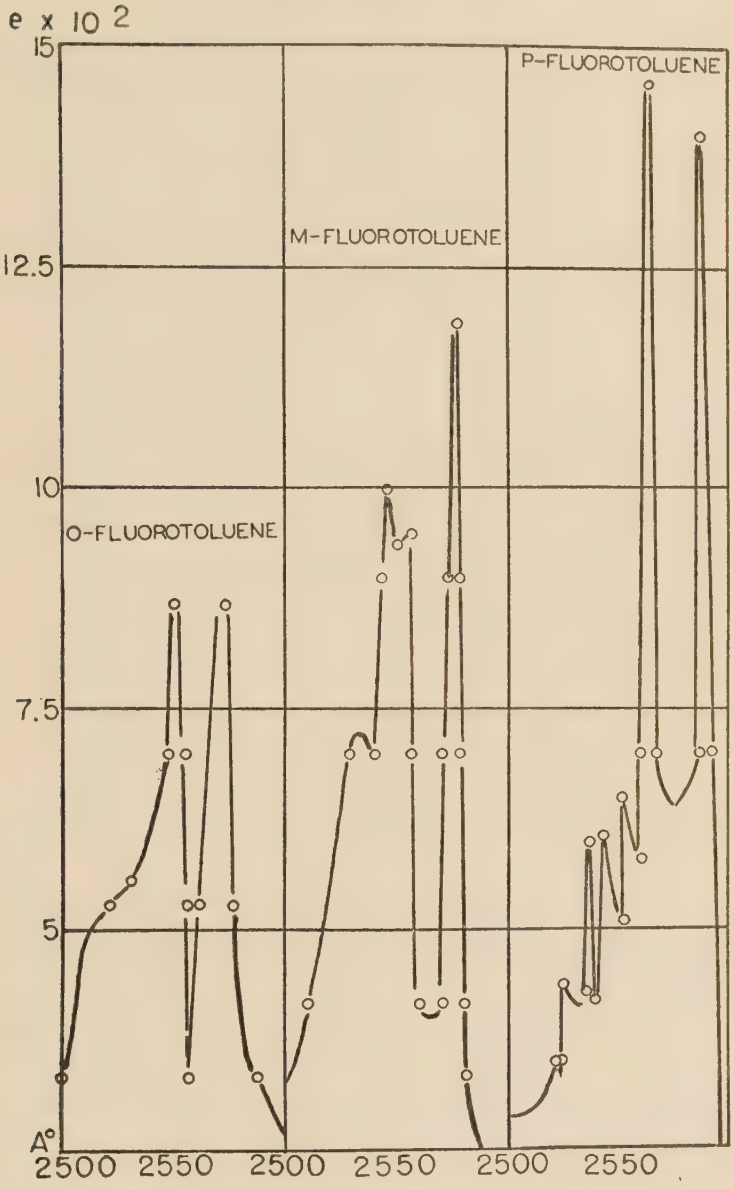
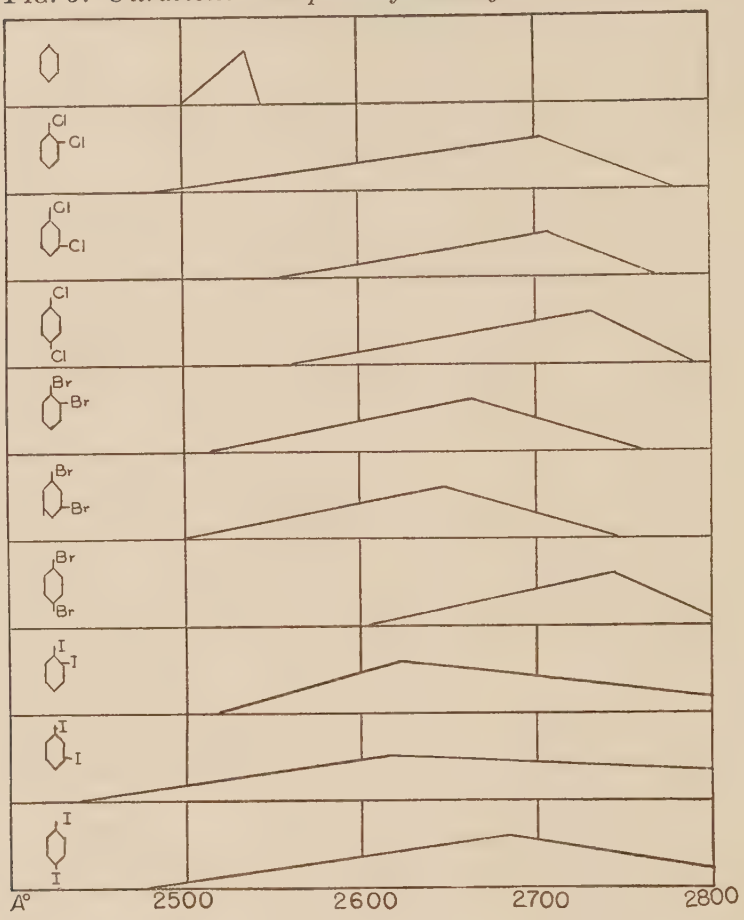


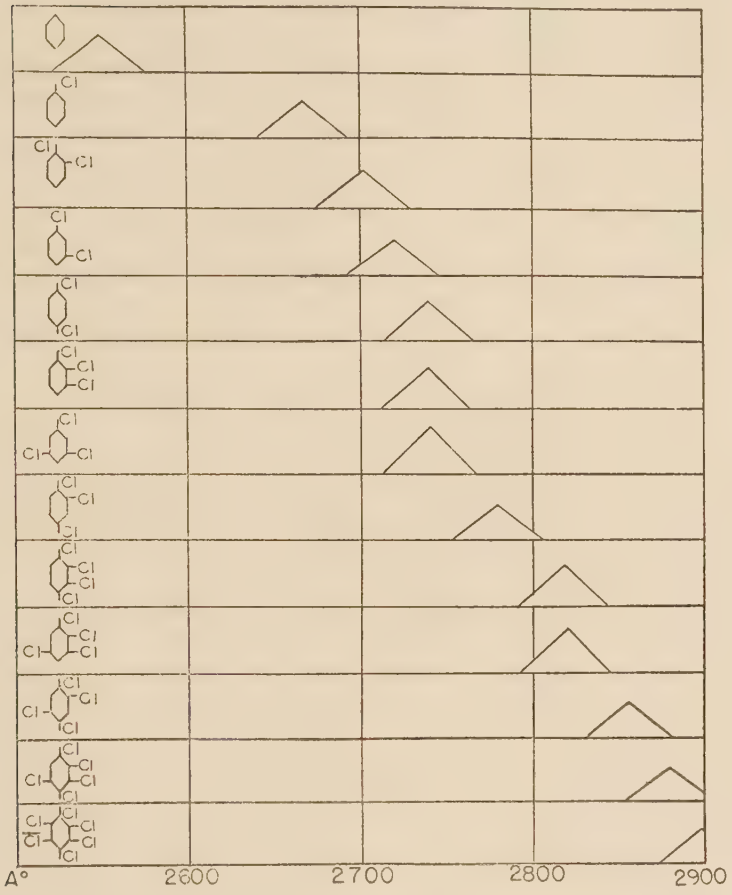
FIG. 5. *Ultraviolet absorption of dihalogenated benzenes*[25].



When the *para* position is filled the band is shifted more to the visible than when the *meta* position is filled. The effect of so filling the *para* position is comparable when either the chloro- or methyl- group is substituted. The effect of increased halogen substitution through the series from the mono- to the hexachlorobenzene is shown in Figure 6[27] in which it is seen

[27] IDEM: *ibid.* **B 19** (1932): 76; KLINGSTEDT, F. W.: *ibid.* **B 20** (1933): 125; MOHLER, H., AND PÓLYA, J.: *Helv. Chim. Acta* **19** (1936): 1222.

FIG. 6. *Effect of increased chlorine substitution on the ultraviolet absorption of benzene*[27].



that the shift to longer wavelengths depends not only on the number of atoms introduced but also on the relative position filled (compare *p*-dichlorobenzene and *sym*-trichlorobenzene). Not only is the *para* position more bathochromic, but it is also characterized by a spectrum of more marked fine structure and of greater strength. The spectrum of the hexachlorinated product has lost practically all the characteristics of aromatic

absorption and approaches the end absorption shown by such compounds as dichloropentane[24].

Mohler and Pólya,[28] Hillmer and Paersch,[29] and Pestemer and co-workers[30] studied the effect of the halogen in various positions in the side chain. The distance from the ring was investigated and seems to indicate that the bathochromic effect and the strength of the absorption follows the order of reactivity of the halogen atom in the same positions:

ring halogen < alkyl halide < side chain halogen

	Iodobenzene	Phenylpropyl iodide	Benzyl iodide
Å	2570	2605	2700
ϵ	616	730	2100

Likewise in a comparison of the spectra of benzyl chloride, benzal chloride and benzotrichloride it is seen that the successive chlorination of the side chain acts as increased substitutions in shifting the absorption of the benzene chromophore toward the visible, and so parallels the halogen reactivity of these compounds.

The effect of substituents on the ultraviolet absorption of biphenyl has been investigated by Rodebush and co-workers[13, 31] who have concluded, in general, that the ultraviolet absorption serves as a sensitive test for restricted rotation where coplanarity is necessary for resonance. The introduction of halogens into the ring of itself has but little effect on the absorption[32] of the benzene ring as was seen in

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[28] Mohler, H., and Pólya, J.: *Helv. Chim. Acta* **19** (1936): 1239.

[29] Hillmer, A., and Paersch, E.: *Z. physik. Chem.* **A 161** (1932): 46.

[30] Pestemer, M., Langer, T., and Manchen, F.: *Monatsh. Chem.* **68** (1936): 326.

[31] Williamson, B., and Rodebush, W. H.: *J. Am. Chem. Soc.* **63** (1941): 3018.

[32] Pestemer, M., and Mayer-Pitsch, E.: *Monatsh. Chem.* **70** (1937): 104.

FIG. 7. *Ultraviolet absorption spectra of para-halotoluenes.* Curve 1: Fluorotoluene. Curve 2: Chlorotoluene. Curve 3: Bromotoluene. Curve 4: Iodotoluene.

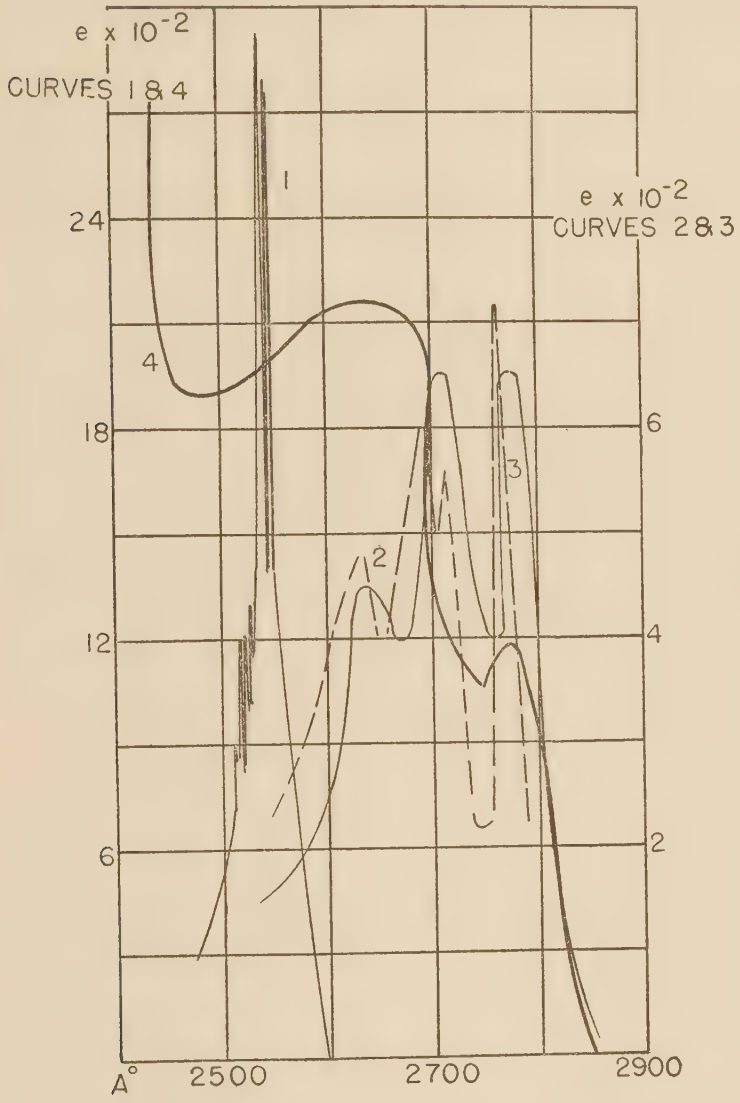
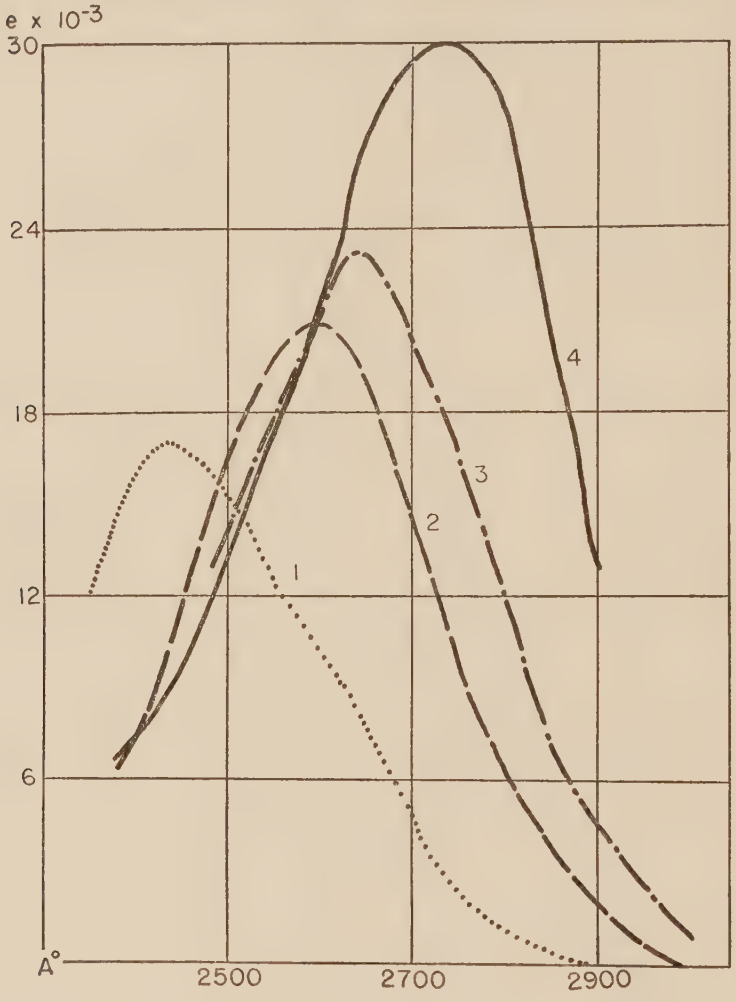


FIG. 8. *Absorption spectra of 4, 4′-dihalodiphenyls.* Curve 1: 4, 4′-difluorodiphenyl. Curve 2: 4, 4′-dichlorodiphenyl. Curve 3: 4, 4′-dibromodiphenyl. Curve 4: 4, 4′-diiododiphenyl.

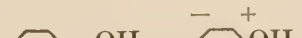


the case of benzene-fluorobenzene, although as the halogen/hydrocarbon ratio becomes greater the absorption band shifts to longer wavelengths. Figures 7 and 8, representing the position and strength of the maxima for the *p*-halotoluenes

and the *p,p'*-dihalodiphenyls, respectively, show that although the maxima for the toluenes are at somewhat longer wavelengths the shift in going from the fluoro-⩾chloro-⩾bromo-⩾iodo- compound is of the same order of magnitude for both series. The difference in the wavelength is qualitatively dependent on the differences in the electromeric polarizability of the halogens[33]. When chlorine is in the 2,2′ positions in biphenyl only end absorption is found, indicating that substitution in this position interferes with the coplanarity of the rings, while in the 4,4′ positions there is no substantial change in the type of absorption.

Oxygen Derivatives

Hydroxyl Compounds. Phenol, the simplest oxygen derivative of the aromatic hydrocarbons, has been the subject of considerable study[34] in various solvents such as heptane, ethanol, methanol, acid, and base. Figure 9 shows the effect of pH on this compound. In non-polar solvents marked fine structure is evident which disappears with increased polarity of the solvent so that the five bands present in heptane fuse into one in methanol with the peak at 2730 Å. Phenolic resonance in acid and alkaline solutions is:



max. 2680 Å

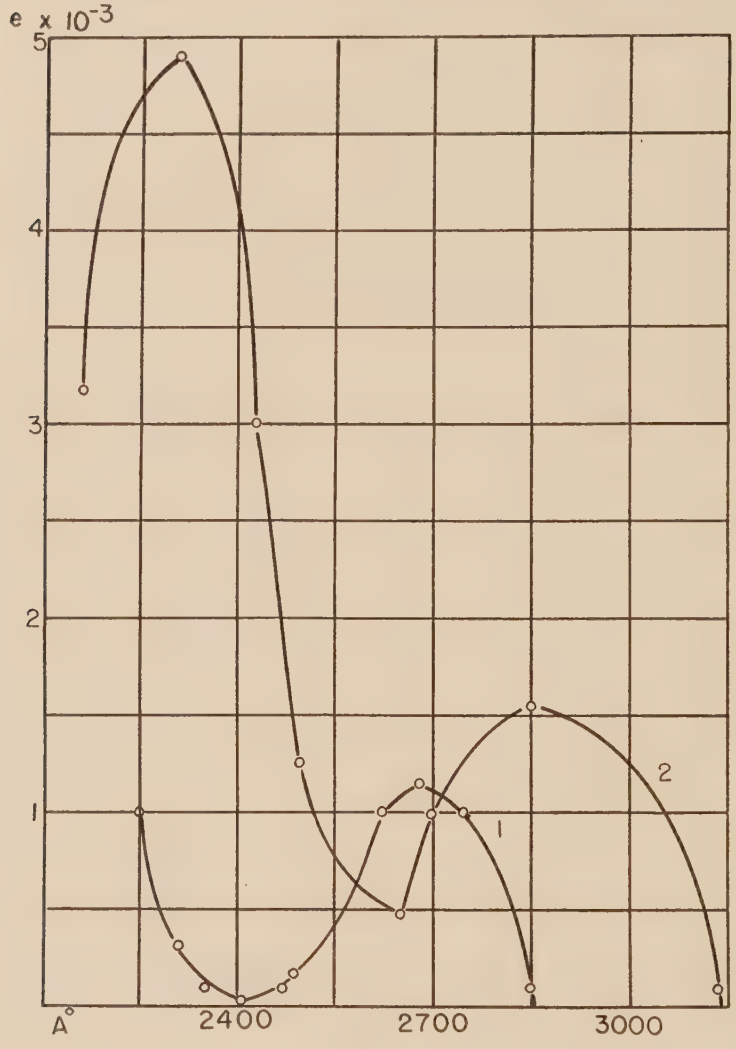
max. 2850 Å

and therefore in a dipolar solvent such as methanol the maximum represents the absorption of a combination of both

[33]Johnson, J. R.: Gilman, H., Organic Chemistry, Chapter 25, 2nd Edition. John Wiley and Sons (1943). New York.

[34]Lauer, K., and Shingu, H.: *Ber. deut. chem. Ges.* **70** (1937): 326; Kepianka, E., and Marchlewski, L.: *Bull. soc. chim.* **35** (1924): 1613; Bodforss, S.: *Ann. Chem.* **534** (1938): 226; Kortüm, G.: *Z. physik. Chem.* **B 42** (1939): 39.

FIG. 9. *Effect of pH on ultraviolet absorption of phenol.* Curve 1: phenol in acid. Curve 2: phenol in base.



resonance pairs, whereas in a non-polar solvent the absorption involves primarily the resonance between the classical Kekulé structures for benzene.

FIG. 10. *Ultraviolet absorption of the cresols.* Curve 1: *p*-cresol. Curve 2: *m*-cresol. Curve 3: *o*-cresol[35].

The effect of the side chain on the hydroxyl influence was studied by Hillmer and Schorning[8]

		λ max., Å	
$C_6H_5CH_2CH_2OH$	2290	2590	2690
$C_6H_5CH_2CH=CHOH$		2590	2690
$C_6H_5CH=CHCH_2OH$	2490		
$C_6H_5CH(OH)CH=CH_2$	2050 2470		

whose data indicate that the saturated phenyl alcohols absorb similarly to benzene in a nonpolar solvent. In the same type of solvent unsaturation in the side chain has varying effects. When unsaturation is β to the ring there is no appreciable effect on absorption as compared with that of the saturated alcohol, but when either the unsaturation or the hydroxyl group is α to the ring the maximum of the band is shifted about 100Å to the shorter wavelengths. This is a shift in the opposite direction to that shown by unsaturation in the same position in propenyl benzene; however, in the alcohols under consideration, the hydroxyl group acts as an unsaturated unit in transmitting vibrations so that the activity of the displacements may be transferred to a more remote atom.

Substitution of other positions of the benzene ring was investigated by Hogness, Sidwell, and Zscheile[35] and the values of the *o*-, *m*-, and *p*-positions are shown in Figure 10. Figure 11 presents the data of Conrad-Billroth[36]. It will be noted that when the hydroxyl and the methyl groups are *para* to each other the maximum is at 2800Å. In the case of 2,5- xylenol not only does the band of the *para* substitution appear but also that of the hydroxyl *ortho* to a methyl group. A comparison of the spectra of propenyl benzene and *para*-propenylphenol shows that from 2600 Å up to 2900 Å the positions of the peaks are the same but the long wave peak of propenyl benzene is shifted to shorter wavelengths by about 40Å, by the introduction of the hydroxyl in the *para* position. The substitution of hydroxy groups on the rings in stilbene[37] causes no appreciable change in comparison with the α, β-dialkylated stilbene.

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[35]Hogness, T. R., Sidwell, A. E., Jr., and Zscheile, F. P., Jr.: *J. Biol. Chem.* **120** (1937): 239.

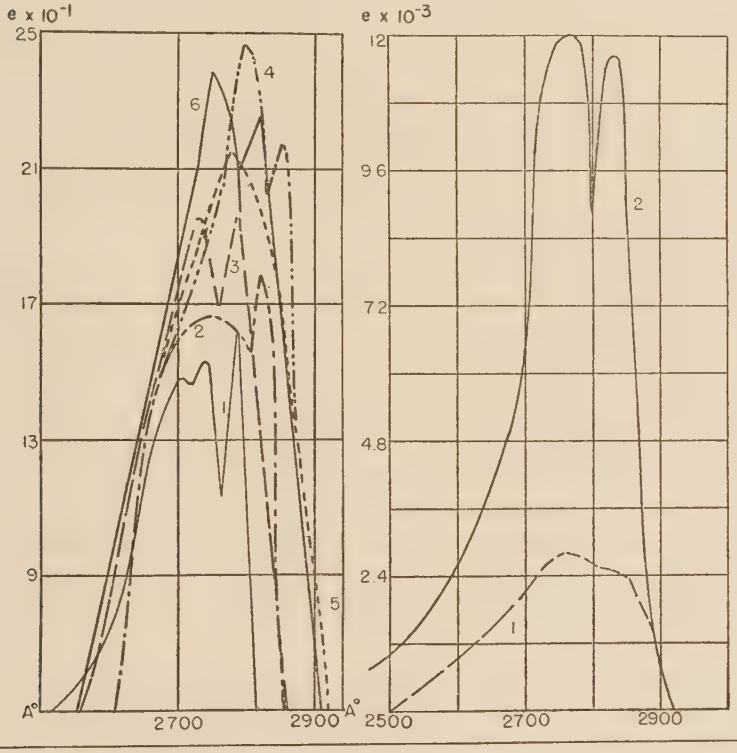
[36]Conrad-Billroth, H.: *Z. physik. Chem.* **B 20** (1933): 222.

[37]Elvidge, W. F.: *Quart. J. Pharm. Pharmacol.* **12** (1939): 347; Hertel, E., and Lührmann, H.: *Z. physik. Chem.* **B 44** (1939): 261.

α, β-dimethylstilbene	4,4′-dihydroxy-α, β-diethylstilbene
λ max. = 2410 Å	λ max. = 2400 Å

The introduction of a hydroxyl group into biphenyl has an effect[38] analogous to that of hydroxyl substitution on the benzene ring alone, if consideration is taken of the steric hindrance resulting in the compound investigated.

FIG. 11. (*Left*). *Ultraviolet absorption of the xylenols*[36]. Curve 1: 2, 3-xylenol. Curve 2: 3, 5-xylenol. Curve 3: 2, 6-xylenol. Curve 4: 3, 4-xylenol. Curve 5: 2, 4-xylenol. Curve 6: 2, 5-xylenol. FIG. 12. (*Right*). *Ultraviolet absorption of catechol* (*Curve 1*) *and resorcinol* (*Curve 2*)[40].



[38] ANCHEL, M., AND BLATT, A. H.: *J. Am. Chem. Soc.* **63** (1941): 1948.

Hodgson[39] in investigating the effect of pH on halogenated phenols reports that with the exception of *m*-bromophenol all *meta*-substituted phenols have a shift in the short wave maximum of approximately the same amount in going from acid to basic solution. The 2700 Å band changes in the following way: Me < OMe < OH. The relative shift in acid solution caused by the introduction of the *meta* substituent is: Me = OH = OMe, while in alkaline solution this effect is: Me < OMe < OH when compared with phenol. In all three cases the halogen effect is proportional to the polarizability of the halogen introduced. The combination of unsaturation and the hydroxyl group is apparently hypsochromic.

The spectra of the dihydroxy benzenes, catechol and resorcinol,[40] are shown in Figure 12. The *para* compound goes so readily into the quinone form that it will be presented with that class. The influence of a side chain in the *para* position to one of the hydroxy groups is given by Hillmer and Schorning[11] who compared pyrocatechol and allylpyrocatechol. In the latter compound the maximum is shifted to slightly longer wavelengths accompanied by a small increase in the absorption strength. The spectra of a number of hydroxy condensed ring compounds were studied by Billroth[41] who found considerable difference in the position of the substituents but could not draw any conclusions.

When the hydroxyl group is alkylated there is very little difference between the resulting ether and the original compound with respect to absorption in non-polar solvents; however, in polar solvents the free hydroxyl group responds more[42]. If two hydroxyl or alkoxyl groups are in the *ortho*

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[39] Hodgson, H. H.: *J. Chem. Soc.* (1943): 380.

[40] Adams, R., Cain, C. K., and Wolff, H.: *J. Am. Chem. Soc.* **62** (1940): 732.

[41] Hua-chih, C., and Conrad-Billroth, H.: *Z. physik. Chem.* **B 20** (1933): 333; see also Bandow, F.: *Biochem. Z.* **301** (1939): 37.

[42] Wessely, F., and Prillinger, F.: *Ber. deut. chem. Ges.* **72** (1939): 629.

position they act as if only one group had been introduced. If the *meta* substituents are alkoxyl groups they are less bathochromic than the *ortho* or *para* substituents but if they are hydroxyl groups then the effect of the *meta* group is the same as that of the *ortho*.

As a special class of hydroxy compounds the quinones may be mentioned[43]. Marchlewski and Moroz[44] studied benzoquinone, α-naphthoquinone, anthraquinone and phenanthraquinone in absolute alcohol and found the following results:

Benzoquinone	2850 Å	($\epsilon = 27700$)
Napthoquinone	3300 Å	($\epsilon = 23400$)
Anthraquinone	3250 Å	($\epsilon = 40000$)
Phenanthraquinone	3250 Å	($\epsilon = 37000$)

The effect of solvent[45] is shown as follows:

Solvent	1-Oxyanthroquinone Wavelength Å			2-Oxyanthroquinone Wavelength Å			
Hexane	4020	3200	2460	3850	....	3060	2750
Benzene	4050	3220	....	3900	3300	3080	....
Ethanol	4080	3290	2500	3840	....	3010	2750
H_2SO_4(conc.)	4160	3100	2660	5000	4080	3210	2890
NaOH	5000	....	2400	4980	3400	3020	2350

From this Lauer and Horio[45] and Schwarzenbach and Suter[46] found that pH affects 2,5-dioxybenzoquinone (1,4) by causing the 3000 Å band to shift toward shorter wavelengths with increasing acidity over the range from pH 10.12 to -0.52. The

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[43] Morton, R. A., and Earlam, W. T.: *J. Chem. Soc.* (1941): 159; Bandow, F.: *Biochem. Z.* **298** (1938): 81; Macbeth, A. K., Price, J. R., and Winzor, F. L.: *J. Chem. Soc.* (1935): 325.

[44] Marchlewski, L., and Moroz, A.: *Bull. soc. chim.* **35** (1924): 473.

[45] Lauer, K., and Horio, M.: *J. prakt. Chem.* **N.F. 145** (1936): 273; *Ber. deut. chem. Ges.* **69** (1936): 130.

[46] Schwarzenbach, G., and Suter, H.: *Helv. Chim. Acta* **24** (1941): 617.

TABLE II. *Ultraviolet Absorption of Benzoyl Acetone in Various Solvents**

Solvent	λmax.	εmax.	λmax.	εmax.	Enol Obs., %	Enol calcd., %
Hexane	2460	5,700	3060	14,800	99.2	98.0
Pentane	2450	5,800	3060	14,600	99.2	98.0
Ether	2450	6,000	3060	14,700	98.1	98.0
Ethanol	2455	6,000	3105	13,800	94.0	92.0
Methanol	2445	6,400	3105	13,450	90.0	89.7
Water	2495	10,300	3100	5,500	32.5	36.7

*Data from Morton, Hassan, and Calloway[47].

5000 Å band did not shift between 10.12 to 3.96, but at pH −0.52 was at 4100 Å. This is to be expected both from consideration of the quinone system and the substituted hydroxy groups.

Carbonyl Derivatives The true carbonyl compounds have been the subject of many studies. Morton, Hassan, and Calloway[47] have tried to relate absorption and constitution of keto-enols. For benzaldehyde and acetophenone[48] the following bands are given:

Benzaldehyde	Å	2440	2805	3280
	ε	16250	1640	20
Acetophenone	Å	2415	2785	3200
	ε	16250	1150	54

Comparing these data with those for aliphatic aldehydes and ketones as well as with benzene, these authors[47] concluded that the absorption is that of the benzoyl group. To test this conclusion they studied benzoyl acetone in solvents of varying polarity. Table II is taken from their work. On the basis of

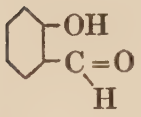
[47]MORTON, R. A., HASSAN, A., AND CALLOWAY, T. C.: *J. Chem. Soc.* (1934): 883.

[48]TASAKI, T.: *Acta Phytochimica* 3 (1927): 259.

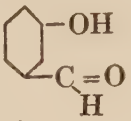
TABLE III. *Ultraviolet Absorption of Ketones**

		λmax., Å	log ε
Acetone	$CH_3\ C\ CH_3$ $\quad\quad\quad

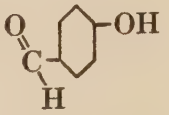
TABLE IV. *Ultraviolet Absorption of Hydroxyl and Alkoxyl Aldehydes and Ketones**



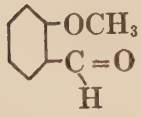
Å	ε
2550	10,000
3250	3,020



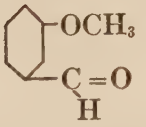
Å	ε
2540	8,100
2930	1,100
3160	2,890



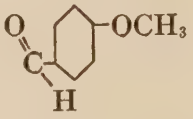
Å	ε
2210	13,800
2840	17,400
3320	178



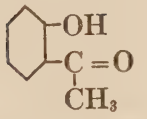
2530	11,700
3195	4,270



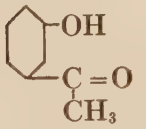
2525	8,320
3145	2,820



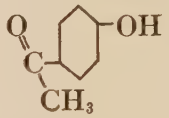
2190	11,800
2770	14,800



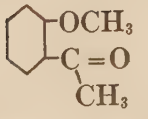
2515	9,330
3270	3,160



....	.....
....	.....



2205	10,720
2760	13,500



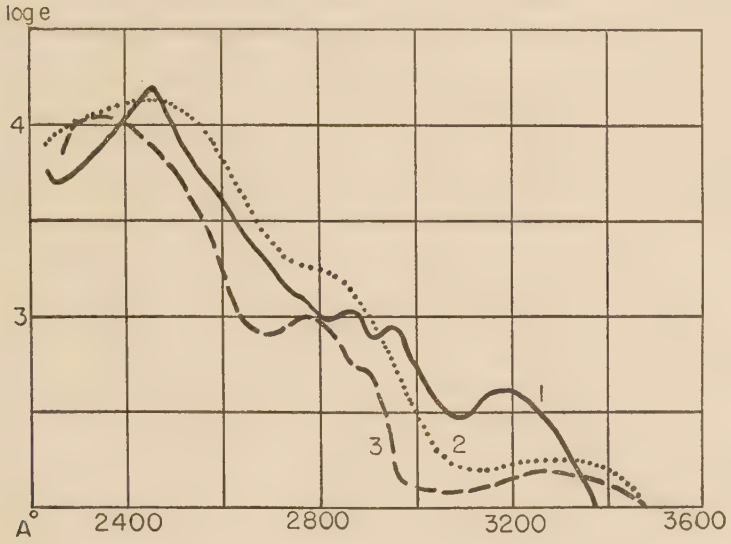
3050	3,800
2460	11,480

*Data from Morton and Stubbs[52].

In a series of hydroxyl and alkoxyl aldehydes and ketones Morton and Stubbs[52] report the data on *o*-, *m*-, and *p*- derivatives given in Table IV. From their work it is seen that as with the xylenes and halotoluenes the *meta*-substituted compounds are not as strongly absorbing as either the *ortho* or *para* products. Apparently the carbonyl attached hydrogen atom is more reactive than the hydroxyl hydrogen.

[52]MORTON, R. A., AND STUBBS, A. L.: *J. Chem. Soc.* (1940): 1347.

FIG. 13. *Effect of substitution on aromatic ultraviolet absorption of carbonyl compounds.* Curve 1: benzoin. Curve 2: desyl-chloride. Curve 3: methyldexoxybenzoin.



The effect of substitution on aromatic carbonyl compounds is shown in Figure 13. It is again seen that the combination of the hydroxyl group and an unsaturated group causes a hypsochromic shift. Although the spectra of aromatic carbonyl compounds where the group is α to the nucleus are characterized by the band at 3100 Å, whereas aliphatic carbonyls absorb at about 2700-2800 Å, in the group benzal acetophenone, dibenzal acetone and cinnamal acetophenone, Rădulescu[53] found that when the carbonyl group is separated by conjugated double bonds the main absorption is still at 3100 Å or longer, thus indicating that the absorption is that of the complete conjugated carbonyl group and not that of the carbonyl affected by the adjacent aromatic ring.

[53] RĂDULESCU, D.: *Ber. deut. chem. Ges.* **64** (1931): 2243; PREISWERK, P., AND ERLENMEYER, H.: *Helv. Chim. Acta* **17** (1934): 329.

Benzoic acid[34] was examined by Kepianka and Marchlewski and found to have an absorption maximum at 2680-2770 Å. Cooke and Macbeth[54] do not find the long wave band as above but report a maximum at 2270Å. However, Henri[55] reports both a long and a short wave absorption band. This is also seen to be the case in the toluic acids examined by Cooke and Macbeth for which bands at about 2300 Å and 2750 Å are reported. In these compounds the short wave peak is probably associated with carboxyl absorption since the saturated aliphatic acids absorb in the neighborhood of 2100 Å. The separation of the ring and the carboxyl group by one carbon atom in phenylacetic acid causes the maxima to shift to shorter wavelengths[50], while the dicarboxylic aromatic acids absorb at longer wavelengths; especially is this the case for terephthalic acid. As is the case with other active groups, increased conjugation results in absorption more to the visible if not impeded by steric hindrance. Smakula[56] finds that as with stilbene the *trans* form absorbs about twice as much as does the *cis* form and at 130 Å toward longer wavelengths.

SUMMARY

1. The absorption strength of the benzene ring is increased by the substituents: alkyl, halogen, hydroxyl, alkoxyl; the substituents being arranged in the ascending order of their effectiveness. In most cases the extinction is greater than for unsubstituted benzene. Förster and Wagner[57] found that the

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[54]Cooke, R. G., and Macbeth, A. K.: *J. Chem. Soc.* (1938): 1408.

[55]Henri, V.: Études de Photochimie. (1919). Paris; see also Tsuzuki, Y.: *Sci. Papers Tokyo Inst. Phys. Chem. Research* **35** (1939): 425; Bednarczyk, W., and Marchlewski, L.: *Bull. intern. acad. polon. sci., Classe sci. math. nat.* **A** (1937): 187.

[56]Smakula, A.: *Angew. chem.* **47** (1934): 657.

[57]Förster, G., and Wagner, J.: *Z. physik. Chem.* **B 37** (1937): 353.

same substitutions on the 3- position in pyrene cause a shift to longer wavelengths, the magnitude of which increases in the order of the above series accompanied by decrease in the extinction.

2. In di-substituted aromatic compounds the position occupied is important in determining the effect of the group. *Ortho* and *para* substitutions are, in general, more effective than *meta* substitutions in bringing about shifts to longer wavelengths. There is also more pronounced fine structure when the *para* position is filled.

3. When active groups are attached through a side chain there is very little activity if the chain is saturated; however, if unsaturation in the chain is conjugated with the benzene ring the distinctive aromatic absorption is lost.

4. Apparently in aromatic compounds the absorption is not that of the component parts of the molecule, but is the result of the entire resonating system, so that it is more difficult to distinguish certain wavelengths as those of specific groupings.

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Original Manuscript Submitted
December 20, 1943

A GROWTH-PROMOTING FACTOR PRODUCED BY ULTRAVIOLET IRRADIATION OF *STICHOCOCCUS BACILLARIS* NAEGELI AND *STICHOCOCCUS FLACCIDUS* (KUTZ) GAY[1]

SISTER CRESCENTIA GIERSCH, C.S.J.[2]

In a preliminary report[3], it was stated that irradiation of *Stichococcus flaccidus* (Kutz) Gay with ultraviolet light produced a factor remaining in the cell-free filtrate which stimulated cell division of the organism. Meier[4] reported that the mutiplication of the *Stichococcus bacillaris* Naegeli is proportional to the intensity of the illumination, and further investigations,[5] indicate that sublethal doses of ultraviolet light stimulate cell division of unicellular green algae. Sperti, *et al.*,[6] and Loofbourow, Dwyer, and Morgan,[7] have shown that injury of *Saccharomyces cerevisiae* by ultraviolet irradiation leads to the production of growth-promoting substances for the organism. Recently the results of Sperti and Loofbourow were further substantiated by the investigations of Cook and

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[1]From the Rose Waller Research Laboratory, Marymount College, Salina, Kansas.

[2]The problem was suggested by Dr. George Speri Sperti and directed by Dr. Elton S. Cook. The spectrographic analysis of the irradiated filtrate was made by Sr. Miriam Michael Stimson, O.P., Siena Heights College, Adrian, Michigan.

[3]Giersch, Sr. C., and Cook, E. S.: *Nature* **148** (1941): 754.

[4]Meier, F. E.: *Smithsonian Misc. Coll.* **92** (1934): 1.

[5]Meier, F. E.: Unpublished investigations summarized in Annual Report Smithsonian Institution. (1937): 105.

[6]Sperti, G. S., Loofbourow, J. R., and Dwyer, Sr. C. M.: *These Studies* **1** (1937): 163.

[7]Loofbourow, J. R., Dwyer, Sr. C. M., and Morgan, Sr. M. N.: *These Studies* **1** (1938): 137.

Cronin[8, 9]. Davidson,[10] by exposing yeast to ultraviolet light, obtained nitrogenous materials in the surrounding medium which stimulated the growth of yeast. Hollaender and Duggar[11] found that cell division in *Escherichia coli* was stimulated by sublethal doses of ultraviolet radiations. Loofbourow and Morgan[12] reported that irradiated filtrates of *Ecsherichia coli* stimulated the growth of *Saccharomyces cerevisiae* while irradiated filtrates of *Staphylococcus aureus* stimulated the growth only at low concentrations.

EXPERIMENTAL

The algae were cultured on medium made by adding 2 per cent agar (Difco) and 2 per cent dextrose (Difco) to Detmer's ⅓ solution and dispensed into test tubes (¾ x 6 in.) or into flat 8-ounce bottles. In preparing the algae for irradiation, the growth from four agar slants or from one bottle was washed off with 60 ml. of Detmer's ⅓ solution, thoroughly mixed, and the suspension then divided into two equal parts. One part was poured into a quartz tube and irradiated with a Sperti Mercolite ultraviolet lamp (42TC) for periods of time ranging from 1.5 to 3 hours at a distance of 15 or 25 cm. This suspension was mechanically stirred during the irradiation period. The second portion, used as the first control, was poured into an ordinary test tube, shielded from the ultraviolet light, and stirred by the same motor as was the irradiated suspension. At the end of the irradiation period, the irradiated and control suspensions were filtered through sterile Berkefeld "N" filters.

For each experiment 5 ml. of a sterile suspension of algae of known cell count per cubic millimeter was distributed to

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[8]Cook, E. S., and Cronin, Sr. A. G.: *These Studies* **3** (1941): 205.

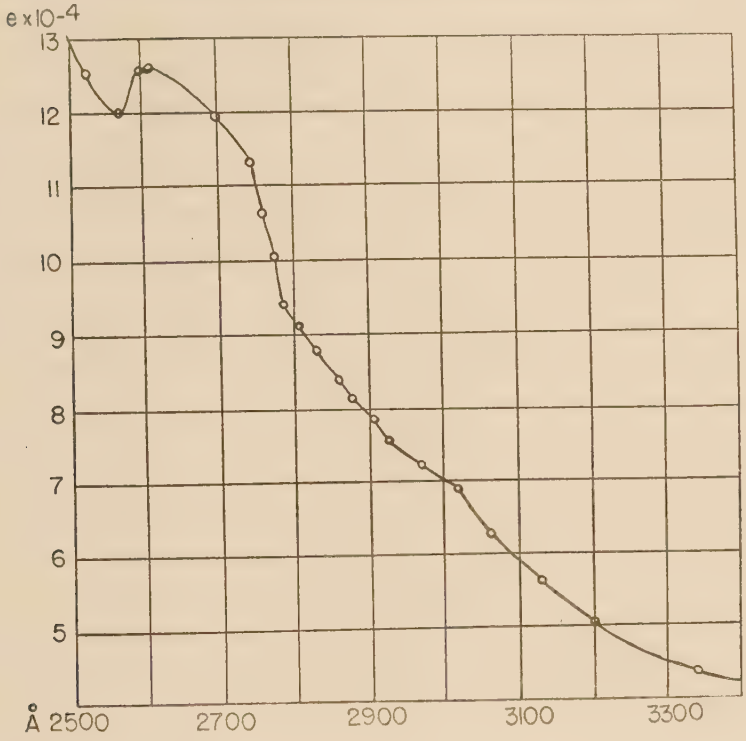
[9]Cook, E. S., and Cronin, Sr. A. G.: *Nature* **150** (1942): 93.

[10]Davidson, J. N.: *Biochem. J.* **34** (1940): 1537.

[11]Hollaender, A., and Duggar, B. M.: *J. Bact.* **36** (1938): 17.

[12]Loofbourow, J. R., and Morgan, Sr. M. N.: *J. Bact.* **39** (1940): 437.

FIG. 1. *Ultraviolet absorption spectrum of the filtrate of ultraviolet irradiated Stichococcus bacillaris.*



each of fifteen sterile test tubes by means of sterile volumetric pipettes. The cell-free filtrate from the irradiated suspension was added to each of five tubes in 2.0 ml. amounts; the cell-free non-irradiated filtrate to each of five other tubes in 2.0 ml. amounts. As a second control, 2.0 ml. of Detmer's ⅓ solution was added to the third set of five tubes.

All cultures were placed on window sills with a northern exposure at room temperature and allowed to grow for 28 days. Temperature variations ranged from 20-23° C. during the winter months, and from 25-30° C. during the summer. Growth, at the end of 28 days, was determined by cell counts

made with a Spencer Bright Line hemacytometer using the area for counting white blood cells.

To determine the specificity of the action of the filtrate, a) the irradiated and non-irradiated filtrates of *Stichococcus flaccidus* were added to cultures of *Stichococcus flaccidus*, b) the irradiated and non-irradiated filtrates of *Stichococcus bacillaris* were added to cultures of *Stichococcus bacillaris*, and c) the irradiated and non-irradiated filtrates of *Stichococcus flaccidus* were added to cultures of *Stichococcus bacillaris*.

In order to determine the stability to autoclaving of the active principle of both the irradiated and non-irradiated filtrates, part of the filtrates from each run were autoclaved at 15 pounds of pressure for 20 minutes, cooled, and then added to cultures of the test organism. The test organism was made up in a known cell count per cubic millimeter and distributed into six additional sterile tubes in the usual manner and these were used for testing the growth-promoting activity of autoclaved irradiated and non-irradiated filtrates.

The spectrum of the irradiated filtrate was determined with a Hilger quartz spectrograph and Spekker photometer (Fig. 1).

RESULTS

No attempt has been made to study the effect of ultraviolet light upon the two unicellular green algae, except in so far as it produces a growth-promoting substance.

Table I shows the effect which the irradiated and non-irradiated filtrates of *Stichococcus bacillaris* had upon cultures of *Stichococcus bacillaris*. In every case in which the irradiated filtrates had been added to the algal cultures there was an increased growth stimulation as compared to cultures to which the non-irradiated filtrate had been added. However, the non-irradiated filtrates showed greater growth-promoting activity than the second control. The rather marked activity

TABLE I. *The influence upon growth of S.bacillaris to which had been added 2.0 ml. of the ultraviolet irradiated and non-irradiated filtrates of S.bacillaris*

	Exp. No.[1]	Mean Yield in Cells/mm.^{3}			Standard Deviation			Mean % Stim. over Controls		Standard % Deviation	
		Irr.	Non-irr.	Controls	Irr.	Non-irr.	Controls	Irr.	Non-irr.	Irr.	Non-irr.
1.5 hr. irradiation at 15 cm. from lamp	1	4290	3230	1830	420	210	470	134	76	23	11
	2	3940	2440	1090	470	480	140	261	124	43	44
	3	9560	7770	4790	1040	1040	410	100	60	22	22
	4	10920	8980	4430	1050	730	660	146	103	24	16
	5	9700	7080	4050	1270	840	300	139	75	31	21
	6	4660	4220	3610	900	310	790	30	17	25	9
	Av.							135	76	28	21
2.5 hr. irradiation at 15 cm. from lamp	7	6020	4450	3140	1145	415	625	92	42	36	13
	8	10360	9790	7550	2620	1960	962	37	30	35	26
	9	11000	10530	6667	1030	1500	1475	65	58	15	22
	Av.							65	43	29	20
3.0 hr. irradiation at 15 cm. from lamp	10	4930	4480	3520	790	690	600	40	24	22	19
	11	3260	3040	2270	320	304	98	44	34	14	13
	12	5290	4000	2690	360	650	435	97	49	13	24
	13	8380	5880	4910	370	250	185	71	20	8	5
	Av.							63	32	14	15
1.5 hr. irradiation at 25 cm. from lamp	14	10400	7540	4300	1270	660	530	142	75	30	15
	15	7670	5410	3960	800	520	510	94	37	20	13
	16	6310	4820	4080	1060	970	1170	55	18	26	24
	Av.							97	43	25	17
2.5 hr. irradiation at 25 cm. from lamp	17	7170	4140	2820	1700	720	220	154	47	60	25
	18	7500	5500	3550	600	720	320	111	55	17	20
	19	8000	7000	3770	550	880	386	112	86	15	21
	20	7500	5550	4140	358	372	250	81	34	9	9
	21	10100	6560	4760	890	270	785	112	38	19	6
	Av.							114	52	24	16
3.0 hr. irradiation at 25 cm. from lamp	22	4730	4240	3770	1000	206	200	25	13	27	6

[1]Five cultures used in each experiment with the exception of No. 3 and 6 in which four cultures were used.

of the non-irradiated filtrates may be due in part to the unavoidable mechanical injury produced during stirring and filtration. This is in agreement with the results of Fardon and Sullivan,[13] and Loofbourow *et al.*[14] who found that mechanically injured cells produce a substance which causes cell proliferation.

In Table II are shown the effects of the filtrates of *Stichococcus flaccidus* upon cultures of *Stichococcus bacillaris*. It will be noted that there is cell proliferation in every case but, as the data in Table I also show, the greater stimulation occurred in those cultures to which the irradiated filtrate had been added. Too, the growth-promoting substance produced by ultraviolet irradiation does not seem to be specific, since there was growth stimulation when the filtrate of irradiated *Stichococcus flaccidus* was added to cultures of *Stichococcus bacillaris*.

Cook and Cronin,[8] have demonstrated that the growth-promoting substance produced by ultraviolet irradiation of yeast, withstood autoclaving at 20 pounds for 20 minutes. Not only does the growth-promoting substance produced by ultraviolet irradiation of algae withstand autoclaving at 15 pounds for 20 minutes, but there are indications that after higher pressures for short periods of time there is an increased growth-promoting activity. Filtrates, irradiated and autoclaved, of *Stichococcus flaccidus* when tested on growth of the same type of alga stimulated growth but since the organism has a tendency to form filaments, accurate counting was difficult. For this reason it was felt that the technical errors were too great to merit inclusion of the data in this report.

Loofbourow, Cook, and Stimson[15] found that the product

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[13] Fardon, J. C., and Sullivan, W. A.: *These Studies* **2** (1938): 39.

[14] Loofbourow, J. R., Cook, E. S., Dwyer, Sr. C. M., and Hart, Sr. M. J.: *Nature* **144** (1939): 553.

[15] Loofbourow, J. R., Cook, E. S., and Stimson, Sr. M. N.: *Nature* **142** (1938): 573.

TABLE II. *The influence upon growth of S.bacillaris to which had been added 2.0 ml. of the ultraviolet irradiated and non-irradiated filtrates of S.flaccidus*

	Exp. No.[1]	Mean Yield in Cells/mm.^{3}			Standard Deviation			Mean % Stim. over Controls		Standard % Deviation	
		Irr.	Non-irr.	Controls	Irr.	Non-irr.	Controls	Irr.	Non-irr.	Irr.	Non-irr.
1.5 hr. irradiation at 15 cm. from lamp	1	8960	5380	4040	440	430	600	122	33	11	11
	2	9680	6430	3980	1310	260	160	143	62	33	7
	3	7790	5750	3530	510	450	200	121	63	15	13
	Av.							129	53	20	10
3.0 hr. irradiation at 15 cm. from lamp	4	7690	5320	4040	720	560	600	90	32	18	14
	5	8390	5880	4190	360	250	190	100	40	9	6
	6	9860	6540	4810	580	280	420	105	36	12	6
	7	7050	6410	4010	700	250	350	76	60	17	6
	Av.							93	42	14	8
2.0 hr. irradiation at 25 cm. from lamp	8	7840	5650	3830	1440	410	290	105	47	37	11
	9	4860	3310	2040	840	510	210	138	61	41	25
	10	5360	4380	2590	340	490	310	106	69	13	19
	11	6310	4390	3240	960	500	560	95	36	29	15
	Av.							111	53	40	17
2.5 hr. irradiation at 25 cm. from lamp	12	6410	4910	4310	1190	720	510	49	14	28	16
	13	9090	6020	4880	2610	600	340	84	25	53	13
	14	6800	4850	2920	750	510	390	133	66	25	17
	15	6410	4690	3160	830	430	150	103	48	26	14
	Av.							92	38	33	15
3.0 hr. irradiation at 25 cm. from lamp	16	8530	7390	4080	550	390	290	109	81	13	10
	17	6960	5840	3970	530	530	480	75	47	14	14
	18	7370	6220	3790	440	410	310	94	64	12	11
	19	8810	7440	4460	380	220	180	97	67	9	5
	Av.							94	65	12	10

[1]Five cultures used in each experiment.

from yeast cells injured with ultraviolet rays has an absorption approximately one thousand times that of the product from ultraviolet-injured algal cells.

SUMMARY

1. Suspensions of *Stichococcus flaccidus* and *Stichococcus bacillaris* were irradiated with ultraviolet for periods of time varying from 1.5 to 3 hours at a distance of 15 or 25 cm. from the lamp.

2. The cell-free filtrate, from both irradiated and non-irradiated suspensions, when added to cultures of algae, stimulated growth. The irradiated filtrate caused the greatest growth increase.

3. The growth-promoting activity of the substance produced by ultraviolet irradiation of algae does not seem to be limited to the species of alga from which the material was derived.

4. The growth-promoting substance is stable to autoclaving at 15 pounds of pressure for 20 minutes.

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PROLIFERATION-PROMOTING PROPERTIES OF HYDROLYZED NUCLEIC ACID[1]

SISTER MARY ELIZABETH ENGLERT, O.P.[2]

In the early studies of crude cell-free filtrates from injured yeast and animal tissue cells, it was found that these preparations were characterized by marked ultraviolet absorption at approximately 2600 Å and that the extinction coefficient paralleled the proliferation potency of these materials[3, 4]. This absorption is characteristic of the purines and pyrimidines of nucleic acid and it was believed that the increased growth might be due to the increased formation of such substances. Chemical findings[4, 5, 6, 7] have confirmed an increase in nucleic acid-like materials when cells are subjected to injuring processes such as ultraviolet irradiations.

In previous work[6, 8] it was found that crude nucleic acid showed growth-stimulating activity, contrary to findings for purified nucleic acid, and that the growth-stimulating material could be extracted from the crude acid with 50 per cent alcohol. On chemical analysis this extract showed the presence of

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[1]From the St. Mary's Dominican College Unit of the Institutum Divi Thomae, New Orleans, Louisiana.

[2]The technical assistance of Misses Gladys Meunier, Marcelle Duvic and Doris Baril is acknowledged.

[3]Loofbourow, J. R., Dwyer, Sr. C. M., and Lane, Sr. M. M.: *Biochem. J.* **34** (1940): 432.

[4]Loofbourow, J. R., Cook, E. S., and Stimson, Sr. M. M.: *Nature* **142** (1938): 573; Cook, E. S., Loofbourow, J. R., and Stimson, Sr. M. M.: *Atti X° congr. intern. chim.* **5** (1939): 26.

[5]Davidson, J. N.: *Biochem. J.* **34** (1940): 1537.

[6]Loofbourow, J. R., Englert, Sr. M. E., and Dwyer, Sr. C. M.: *Nature* **148** (1941): 113.

[7]Loofbourow, J. R., and Joyce, L.: *Nature* **148** (1941): 166.

[8]Englert, Sr. M. E.: Report, Third General Conference of the Institutum Divi Thomae, 1942.

TABLE I. *Effect on Yeast Growth of Nucleic Acid and Derivatives Prepared from Irradiated and Non-irradiated Yeast*

Concentration material tested mg. per ml.	Yeast Growth in Milliliters†				
	Acid	Extract	Residual Acid	Hydrolyzed Acid‡	
				A*	B
	Irradiated Yeast				
1.0	0.025	0.055	0.010	0.055	0.035
0.5	0.025	0.036	0.010	0.045	0.028
0.25	0.023	0.024	0.010	0.042	0.026
0.125	0.020	0.018	0.010	0.042	0.025
0.063	0.018	0.016	0.010	0.035	0.023
Control	0.013	0.010	0.010	0.026	0.011
	Non-irradiated Yeast				
1.0	0.015	0.036	0.010	0.028	0.074
0.5	0.013	0.030	0.010	0.026	0.050
0.25	0.013	0.026	0.010	0.022	0.024
0.125	0.013	0.023	0.010	0.020	0.022
0.063	0.012	0.021	0.010	0.020	0.017
Control	0.010	0.013	0.010	0.013	0.013

†Measured in Hopkins tubes after 24 hours incubation at 30° C.

‡Crude Guanosine fraction, A; Filtrate containing Adenosine-cytidine-uridine, B.

*Volume determined after 48 hours incubation.

adenine, guanine, pentose, and nitrogen, but no phosphorus. The absence of phosphorus suggested the possibility that the active principle in the extract might be a nucleoside, formed during the preparation of the nucleic acid. The present paper describes a preliminary study of the proliferation-promoting activity of the hydrolytic products of nucleic acid.

Several batches of yeast nucleic acid were prepared from irradiated and non-irradiated yeast by the method of Johnson and Harkins,[9] modified in a few details. The acids were extracted with 50 per cent alcohol to remove growth-stimulating substances which are known to contaminate crude

[9]JOHNSON, T. B., AND HARKINS, H. H.: *J. Am. Chem. Soc.* **51** (1929): 1779.

nucleic acids. The residual acids were then hydrolyzed with ammonium hydroxide under pressure[10] and the hydrolytic products separated into two fractions, one containing adenosine, cytidine and uridine, and the other guanosine.

The acids, extracts, residual acids, and hydrolytic fractions were tested for growth-stimulating activity on cultures of yeast grown in Reader's medium[11] in 50 ml. Erlenmeyer flasks at 30° C., and the yeast populations after 24 hours were determined by centrifuging the cultures in Hopkins tubes. The results are summarized in Table I.

The acids from non-irradiated yeast showed only slight growth-promoting activity. Some samples had practically no activity, indicating that these particular preparations were comparatively free from growth contaminants.

However, when the 50 per cent alcoholic extracts of these fairly pure samples were tested for growth-stimulating activity, they showed a growth potency that was 1.8 times that of the control in a concentration of 0.125 mg. material tested per ml. of culture. In a concentration of 1.0 milligram per ml. of culture, the yeast growth was 2.8 times that of the control. These extracts contained nitrogen but no phosphorus or sulphur. They gave positive Molisch, tryptophane, and Jossel tests, but were negative for tyrosine and murexide. To be certain that the alcohol contained no growth factor, controls were run with the alcohol that was used for extraction. These controls gave the same results as the non-alcoholic ones previously used.

As would be expected, the residual acids after extraction exhibited no growth-stimulating properties.

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[10]Levene, P. A., and Bass, L. W.: Nucleic Acids. Chem. Cat. Co., New York, 1931.

[11]Reader, V.: *Biochem. J.* **21** (1927): 901.

The hydrolytic fractions of these residual acids showed growth-promoting properties. In a concentration of 1.0 mg. per ml., some adenosine fractions showed a growth potency five times greater than that of the control. The guanosine fractions in the same concentration showed a potency twice that of the control.

The crude acids from irradiated yeast contained less protein than those from non-irradiated yeast, as determined by the biuret test using the depth of color as an indication of the amount of protein present. The extracts of these acids in concentrations of 0.5 – 1 mg. per ml. showed greater stimulating powers than the extracts of nucleic acids prepared from non-irradiated yeast, but in the lower concentrations these extracts were not superior to those from the non-irradiated acids.

The residual acids after extraction had no growth-stimulating powers; but when hydrolyzed, growth-promoting factors again were present. With the adenosine fraction, in a concentration of 0.125 mg. solid material per ml. of culture, the yeast growth was almost twice that of the control, and in a concentration of 1 mg. per ml., the yeast growth was 2.5 times that of the control. The guanosine fraction from the hydrolysis of nucleic acid prepared from irradiated yeast showed practically the same growth-promoting powers as the guanosine fraction from nucleic acid prepared from non-irradiated yeast.

These results are in general agreement with findings published by Cook, *et al.*[12] on the effects of adenosine and guanosine on the proliferation of yeast.

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[12]Cook, E. S., Cronin, Sr. A. G., Kreke, C. W., and Walsh, Sr. T. M.: *Nature* **152** (1943): 474.

EXPERIMENTAL

Yeast Nucleic Acid. One pound (458.5 gm.) of Fleischmann's bakers' yeast was washed with distilled water until it was starch-free. The starch-free yeast was suspended in 1.5 liters of distilled water and cooled to 0° C. A solution of 150 gm. of sodium hydroxide in 450 ml. of water, also cooled to 0° C., was added to the yeast suspension and the volume made up to 2.5 liters. The mixture was allowed to stand at 0° C. for 4–5 hours, after which time glacial acetic acid was added until the solution was acid to litmus paper (pH 7). The mixture was permitted to stand overnight and the yeast dregs removed by centrifuging. Ninety-five per cent alcohol was added to the clear supernatant liquid to 10 per cent alcohol by volume, and the liquid was permitted to stand for a few minutes. The slight precipitate which formed was filtered off and concentrated hydrochloric acid was added to the filtrate until it was acid to Congo red (pH 3.6). Alcohol was again added to make a solution containing 50 per cent alcohol by volume. The nucleic acid appeared immediately as a white flocculent precipitate. The mixture was allowed to stand overnight in the refrigerator, after which time the nucleic acid was removed by centrifuging. The acid was washed three times with 95 per cent alcohol in the centrifuge bottles. It was then filtered with suction and placed in a desiccator to dry. The yield of a white product, practically protein-free, as indicated by the biuret test, was 9–9.5 gm.

In the preparation of nucleic acid from irradiated yeast, the starch-free yeast suspended in 1.5 liters of distilled water was irradiated with ultraviolet light (Sperti lamp) for 24-27 hours, or until approximately 50 per cent of the cells were killed, as indicated by methylene blue staining. The yield of nucleic acid was 12-13 gm. per pound of baker's yeast.

Hydrolysis of Nucleic Acid. Ten gm. of yeast nucleic acid was dissolved in a solution of 10 ml. of concentrated ammonium hydroxide in 50 ml. of water and the solution heated in an autoclave at 145° C. (inner temperature) for 3.5 hours. When the reaction mixture was cooled, it solidified to an apparently homogeneous jelly. After it was stirred, the crude guanosine was easily filtered off, and washed once with water.

Preparation of Hydrolytic Fractions. The crude guanosine was dissolved in boiling water and titrated with dilute sulfuric acid until acid to litmus paper. The volume was then made up to contain 25 mg. solid material per ml. of solution. This solution was diluted with distilled water to the various concentrations used.

The filtrate from the crude guanosine was titrated with dilute sulfuric acid until acid to litmus paper and the volume made up to contain 25 mg. of solid material per ml. of solution. Dilutions of this solution were made as in the guanosine fraction.

Growth Assays. *Saccharomyces cerevisiae*, grown from a single cell isolated from Fleischmann's bakers' yeast, was washed from 72-hour slants on Sabouraud's medium and made up in sterile Reader's medium so that 30.7 ml. of yeast suspension deposited 0.05 ml. yeast when centrifuged in Hopkins tubes. (One ml. of this suspension contained approximately 0.747 mg. yeast, dry weight.) One ml. of this suspension was added to each 50 ml. Erlenmeyer flask containing 23 ml. of Reader's medium and one ml. of material to be tested. The control flasks contained one ml. of distilled water in place of the ml. of material to be tested. The cultures were incubated without shaking at 30° C. for 24 hours, after which time they were centrifuged in Hopkins tubes to determine the yeast populations.

Extracts. Eight to 10 gm. of crude nucleic acid was extracted three times with 50 ml. of warm 50 per cent alcohol. The

combined extracts were concentrated at 50° C. under reduced pressure to 25 ml., filtered, and diluted to the various concentrations used.

SUMMARY

A preliminary study of the proliferation-promoting activity of the hydrolytic products of nucleic acid has been made. The results of the experiments suggest that the proliferation-promoting activity of products from injured cells is partly due to these hydrolytic products. Chemical and spectrographic evidence indicates the possibility of the identification of these active factors with the nucleosides, guanosine and adenosine.

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January 8, 1944

EFFECT OF YEAST EXTRACT ON THE GROWTH AND RESPIRATION OF *AZOTOBACTER CHROOCOCCUM*

SISTER M. PETRONELLA SCHROEDER, C.PP.S.

YEAST extracts which affect the growth of bacteria have been described in previous issues of these Studies[1] and elsewhere[2]. One of these extracts has now been studied in relation to the growth, respiration, and nitrogen content of the cells of the non-symbiotic organism, *Azotobacter chroococcum*, important in agronomy.

This organism is rich both in the number and content of metabolic enzymes[3]. Furthermore, its high vitamin content—biotin,[4] nicotinic acid,[5] pantothenic acid,[6] and riboflavin[7] —justifies efforts to improve its production. Lee and Burris[8] have reported large scale cultures of this organism in liquid media. The present paper reports the use of a yeast extract to produce large yields on solid media. The study includes investigation of the nitrogen content and respiration of the cells as affected by the extract.

EXPERIMENTAL

Organisms. The culture of *Azotobacter chroococcum* No. 40 was obtained through the courtesy of Mr. F. B. Smith of the Agricultural College of the University of Florida. When grown in a liquid or on a solid medium of inorganic salts to which

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[1]LOOFBOUROW, J. R., AND MORGAN, SR. M. N.: *These Studies* **2** (1938): 113.

[2]LOOFBOUROW, J. R., AND MORGAN, SR. M. N.: *J. Bact.* **39** (1940): 437.

[3]BURK, D., AND BURRIS, R. H.: *Ann. Rev. Biochem.* **10** (1941): 587.

[4]SHULL, G. M., HUTCHINGS, B. L., AND PETERSON, W. H.: *J. Biol. Chem.* **142** (1942): 913.

[5]SNELL, E. C., AND WRIGHT, L. D.: *J. Biol. Chem.* **139** (1941): 675.

[6]STRONG, F. M., FEENEY, R. E., AND EARLE, A.: *Ind. Eng. Chem. Anal. Ed.* **13** (1941): 566.

[7]SNELL, E. C., AND STRONG, F. M.: *Ind. Eng. Chem. Anal. Ed.* **11** (1939): 346.

[8]LEE, B., AND BURRIS, R. H.: *Ind. Eng. Chem.* **35** (1943): 354.

one per cent mannitol was added as a carbon source, this culture produced a gum which interfered with the separation of the organisms from the liquid and with their being washed off the solid medium. Thus, the first attempts at preparing a "resting cell" preparation of the organism failed. Since this gummy substance is an araban,[9] mannitol was omitted from the media and 0.25 per cent of a crude aqueous alcoholic yeast extract[10] was added. There resulted a luxuriant growth of organisms which produced little or no gum and which could be easily separated from a liquid medium by centrifuging, and from a solid medium by washing. Furthermore, when these bacteria subsequently were grown on a medium containing both one per cent of mannitol and yeast extract in concentrations greater than 0.25 per cent, they grew without the gummy covering.

Resting Cell Suspension. "Resting cells," as defined by Quastel and Whetham,[11] are cells which have been centrifuged, washed with saline, and finally well aerated. Such cells in the absence of available nitrogen do not proliferate and responses to experimental conditions are not complicated by additional factors involved in growth. The method used in preparing the "resting cell" suspension was similar to that described by Wilson[12]. The organisms were grown on a solid medium, Medium A, of the following composition:

	gm.
K_2HPO_4	1.1
KH_2PO_4	0.3
NaCl	0.2
$MgSO_4.7H_2O$	0.2
$CaSO_4.2H_2O$	0.1
H_2O	1000.0
Agar	30.0
Yeast extract	2.5

[9]Salle, A. J.: Fundamental Principles of Bacteriology. McGraw-Hill, New York. 1943, p. 53.

[10]Cook, E. S., Kreke, C. W., and Nutini, L. G.: *These Studies* **2** (1938): 23.

[11]Quastel, J. H., and Whetham, M. D.: *Biochem. J.* **18** (1924): 519.

[12]Wilson, P. W.: *J. Bact.* **35** (1938): 601.

A 48-hour culture was washed off the surface of this medium with 50 ml. of an inorganic salt mixture solution, referred to as Medium B, into an Erlenmeyer flask provided with sterile glass beads. After thorough shaking to break up any clumps, the suspension of organisms was filtered through glass wool, centrifuged, washed a second time, and then suspended in Medium B, of the following composition:

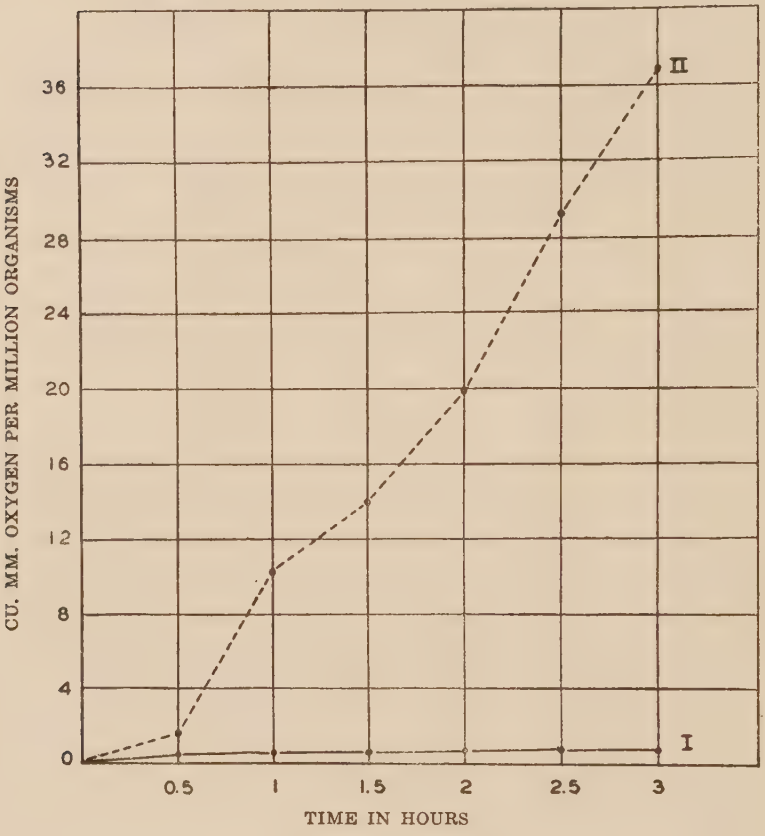
	gm.
K_2HPO_4	1.5
KH_2PO_4	0.3
NaCl	0.2
$MgSO_4.7H_2O$	0.2
$CaSO_4.2H_2O$	0.1
H_2O	1000.0

This "resting cell" suspension of *Azotobacter chroococcum* was kept at approximately 3° C. in a sterile cotton-stoppered Erlenmeyer flask. Before use the culture was shaken well and allowed to come to room temperature. One ml. of this suspension contained 4.92 mg., dry weight, of the organism. This suspension was used for the growth and respiration experiments reported in this paper. The activity of this "resting cell" suspension of *Azotobacter chroococcum* remained fairly constant as determined by periodic endogeneous respiration tests.

Endogeneous Respiration Determination. A comparison was made of the endogeneous respiration of "resting cells" grown on Medium A, and on Medium A in which 1 per cent mannitol had been incorporated. This comparison was made on the basis of equal numbers of cells rather than on a weight basis because the gummy covering of the organisms grown in the presence of mannitol increases the apparent dry weight. The results, shown in Fig. 1, are in agreement with those of Wilson[12] and of Burris and Wilson[13] who investigated the respiratory

[13] Burris, R. H., and Wilson, P. W.: *Proc. Soc. Exptl. Biol. Med.* **45** (1940): 721.

FIG. 1. *Endogenous Respiration of Azotobacter chroococcum.*



Curve I. Organisms previously grown on Medium A; *Curve II.* Organisms previously grown on Medium A plus 1 per cent mannitol.

activity of *Rhizobium trifolii* 209, a symbiotic nitrogen-fixing bacterium producing a gummy covering.

Effects of Yeast Extract on Growth and Respiration. The growth experiments were made in Roux flasks on solid media consisting of the following basal medium, Medium C, to which varying concentrations of the yeast extract were added. Medium C has the following composition:

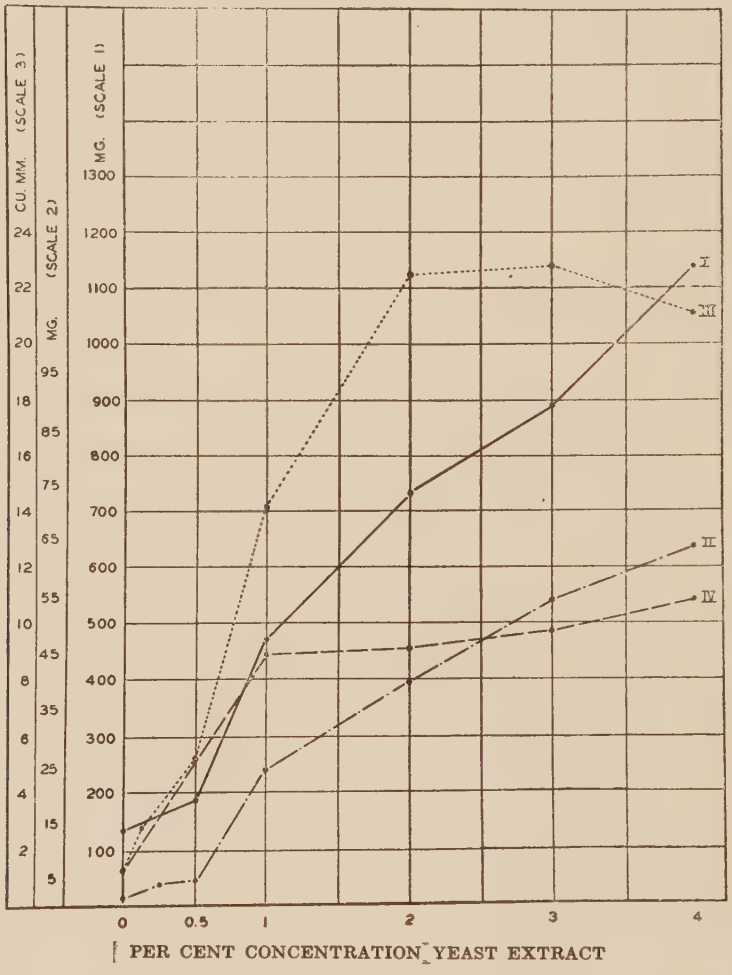
	gm.
Mannitol	10.0
K_2HPO_4	0.5
$MgSO_4$	0.2
NaCl	0.1
$CaCO_3$	3.0
H_2O	1000.0
Agar	30.0

The agar content was increased from the usual 2, to 3 per cent to facilitate the removal of the cells from the surface of the medium. One ml. of the "resting cell" suspension was used in seeding the medium which had been autoclaved at 15 pounds for 20 minutes and had been allowed to harden. The cultures were incubated at room temperature (25° C. ∓ 5°) for four days after which the crops were harvested in the following manner: The organisms were washed from the surface of the media containing each of the various concentrations of the yeast extract with 50 ml. sterile distilled water and transferred to an Erlenmeyer flask. An additional 50 ml. of sterile distilled water was used to rinse the surface of the medium and was added to the first washing. The flasks were shaken well to make even suspensions of the organisms. To determine growth, 50 ml. from each flask containing the suspensions were transferred to tared evaporation dishes, and these were then placed in the drying oven for 24 hours. The suspension remaining in the flasks was retained for oxygen-uptake determinations.

Respiration was measured by the direct Warburg method[14]. The temperature of the manometer flasks was maintained at 30° C. throughout the experimental period of 3 hours. Dry weight determinations for the calculation of the oxygen-uptake values were made by drying aliquot portions of the suspensions at 100° C. and deducting the weight of the salts in the suspending solution. The respiration was measured: *a*) of distilled water suspensions of cells which had been grown

[14]Dixon, M.: Manometric Methods. 2nd ed. Macmillan, New York. 1943.

FIG. 2. *Effect on Azotobacter chroococcum of incorporation in media of varying concentrations of yeast extract.*



Curve I. Growth—dry wt. in mg. (Scale 1); *Curve II.* Nitrogen yield—in mg. (Scale 2); *Curve III.* Respiration of proliferating suspension—oxygen uptake in cu. mm./mg. dry wt./hr. (Scale 3); *Curve IV.* Oxygen uptake of cells from stock culture as affected by addition of varying concentrations of yeast extract to respirometer flasks.

TABLE I. *Relation of Concentration of Yeast Extract to Percentage of Cellular Nitrogen in Azotobacter Chroococcum*

Per cent concentration of yeast extract	0.0	0.5	1.0	2.0	3.0	4.0
Per cent nitrogen in cell	0.78	2.48	5.15	5.24	5.95	6.2

on media containing varying concentrations of yeast extract as described in the preceding paragraph; and *b*) of "resting cells" suspended in Medium B to which varying concentrations of yeast extract were added in respirometer flasks. Total nitrogen was determined with the semimicro-Kjeldal method by Miss Julitta Luetmer.

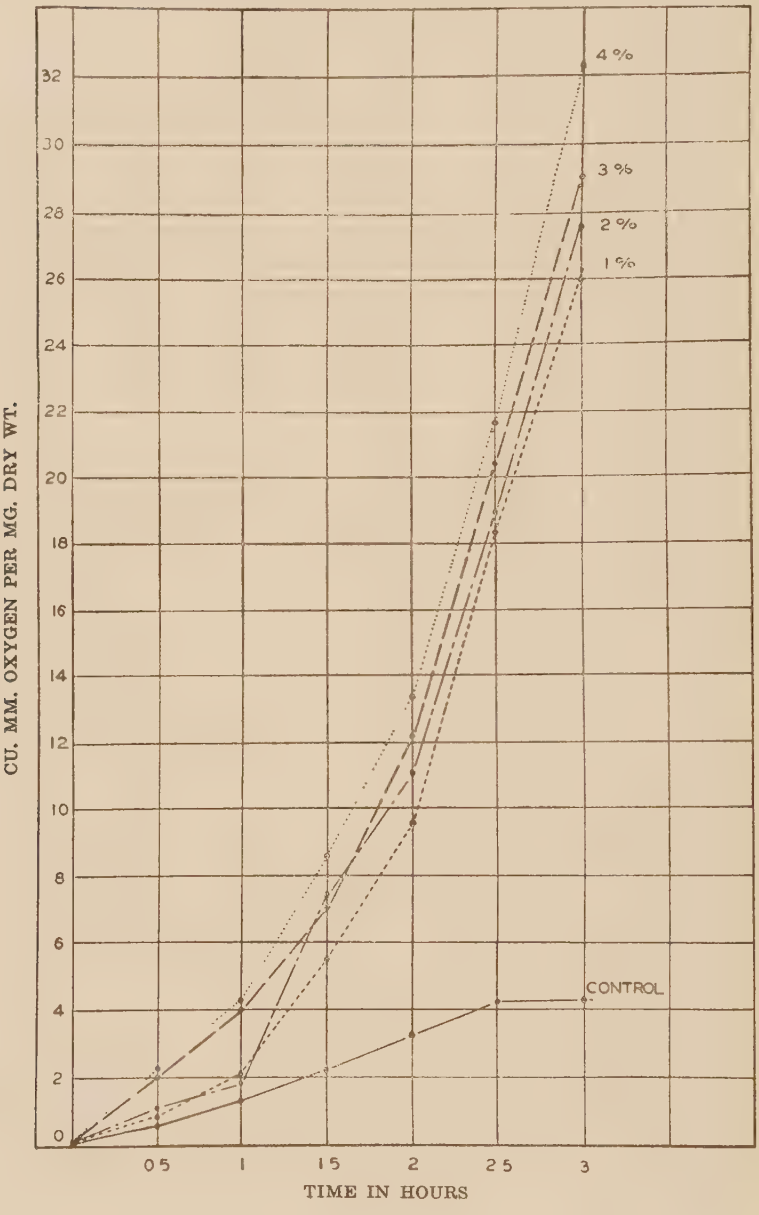
Curves I, II, and III in Fig. 2 present the data on growth, respiration, and nitrogen content. An approximate proportionality exists between the amount of extract incorporated and the crop. It will be noted that the addition of 1, 2, 3, and 4 per cent yeast extract produced marked increases in the total crop, 248, 437, 557 and 736 per cent, respectively.

Curve II shows an increase in total nitrogen (in mg.) with an increase in the total crop. This is of interest because Burk[15] noted that the extracellular ammonia observed in his own and in previous investigations was derived in all probability entirely from the decomposition of normal cell nitrogen and not, in any measurable quantity, by direct synthesis from free nitrogen. The fact that increasing the amount of yeast extract also increases the percentage of nitrogen in the organism as well as the total nitrogen is not shown by the graph. These pertinent data are given in Table I.

Curve III represents the oxygen-uptake of distilled water suspensions of the proliferating organisms calculated from the data for a 3-hour experimental period. It is seen that the

[15]BURK, D., AND HORNER, C.: *Soil Science* **41** (1936): 81.

FIG. 3. *Oxygen uptake of Azotobacter chroococcum in presence of varying concentrations of yeast extract.*



respiration of such suspensions of *Azotobacter chroococcum* is markedly increased when the organisms are grown in media containing up to 2 per cent of yeast extract. Further increase of yeast extract content has no effect on the oxygen-uptake, whereas it continues to exert an effect on total crop and nitrogen content.

Curve IV presents data on the oxygen-uptake of the "resting cell" suspension in Medium B, described earlier in the paper, as affected by the addition of varying amounts of yeast extract to the respirometer flask. These values are calculated from a 3-hour oxygen-uptake. It is seen that 1 per cent of yeast extract greatly increases the oxygen-uptake, but that higher concentrations have relatively little effect. The details of the time-respiration data, the basis of Curve IV, are presented in Figure 3. The information obtained in the current experiment does not indicate to what extent the increase in oxygen-uptake with time is a function of increased proliferation during the respiration period, since the dry weight determinations were made on the initial suspension and not on the suspension at the conclusion of the experiment or at any period during the experiment. The control curve in Fig. 3 shows a steady rate of oxygen-uptake, whereas the experimental curves, after a lag which is greatest with the lowest concentrations, show a great acceleration of rate; this suggests either that increased proliferation during the 3-hour period is an important factor in the increased respiration, or that there is a lag period before the full extent of the stimulation becomes evident. Other experiments, similar to the foregoing, in which the number of cells was counted at the beginning and at the conclusion of the experimental period of respiration indicate that increased proliferation during the initial 3-hour period is responsible for the increased rate of respiration.

SUMMARY

1. A study was made of the growth, respiration, and cellular nitrogen of *Azotobacter chroococcum* in relation to the varying concentrations of an alcoholic yeast extract incorporated in the growth medium.

2. Increasing the content of yeast extract in the medium to levels ranging from 0.5 to 7 per cent caused:

a) An increase in growth of the organism as determined by dry weight which was proportional to the percentage of yeast extract

b) An increase in total cellular nitrogen proportional to the percentage of yeast extract

c) An increase in the percentage of nitrogen in the cell as the amount of yeast extract was increased

d) An increase in the oxygen-uptake of a suspension of proliferating cells with incorporation of yeast extract up to 2 per cent: beyond this concentration there was no greater effect.

3. Addition of yeast extract to suspensions of cells in the respirometer flasks increased the oxygen-uptake which was probably due chiefly to increased proliferation induced by the extract during the respiration period.

Original Manuscript Submitted
March 5, 1944

EFFECT OF *PARA*-AMINOBENZOIC ACID AND OTHER SUBSTANCES ON YEAST RESPIRATION AND ON THE RESPIRATORY-DEPRESSANT ACTION OF PHENYLMERCURIC NITRATE

ELTON S. COOK AND SISTER THOMAS MARY WALSH, O.P.

PREVIOUS papers have demonstrated that yeast extracts can antagonize the inhibitory effects of phenylmercuric nitrate on the respiration[1] and growth[2] of rat skin, on the growth and respiration of yeast,[3] and on the growth of molds[4] and of *Staphylococcus aureus*[1, 5]. Kreke and Suter,[6] investigating the ability of yeast extracts to increase the respiration of yeast, found that the extracts would counteract the respiratory-depressant effect of potassium cyanide and sodium azide but not of urethane or amyl alcohol. Yeast extracts do not offset the respiratory depression caused by local anesthetics[7]. From these findings, together with the observation that the yeast extracts stimulate the action of the iron porphyrin enzymes, peroxidase[8] and catalase,[9] it has been suggested that the extracts may exercise their respiratory effects by action on the oxidase end of the respiratory chain, perhaps by furnishing

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[1]COOK, E. S., KREKE, C. W., EILERT, SR. M. R., AND SAWYER, M. A.: *Proc. Soc. Exptl. Biol. Med.* **50** (1942): 210.

[2]THOMAS, G. W., FARDON, J. C., BAKER, S. L., AND COOK, E. S.: *J. Am. Pharm. Assoc.* **34** (1945): 143.

[3]COOK, E. S., AND KREKE, C. W.: *Proc. Soc. Exptl. Biol. Med.* **53** (1943): 222.

[4]COOK, E. S., AND KREKE, C. W.: *Nature* **146** (1940): 688.

[5]THOMAS, G. W., AND COOK, E. S.: Unpublished data.

[6]KREKE, C. W., AND SUTER, SR. M. ST. A.: *J. Biol. Chem.* **160** (1945): 105.

[7]COOK, E. S., AND MCDEVITT, SR. M. OF L.: *These Studies* **4** (1945): 107.

[8]KREKE, C. W., AND BARTLETT, SR. M. D.: *These Studies* **4** (1945): 1; 15.

[9]KREKE, C. W., BARTLETT, SR. M. D., AND SMALT, SR. M. A.: *J. Biol. Chem.* **158** (1945): 469.

iron complexes which might function as prosthetic groups, or by furnishing autoxidizable substances which carry oxygen directly to hydrogen acceptors.

Since it is not known how phenylmercuric nitrate exerts its respiratory-depressing effect, it is not certain that yeast extracts may antagonize it in the manner suggested above. In fact, some of the earlier evidence was interpreted as indicating a possible interaction between the germicide and an active substance in the extract[1]. It is well known that yeast extracts antagonize the bacteriostatic action of sulfanilamide and that the antagonism is largely due to *p*-aminobenzoic acid[10] and possibly to other substances as well, such as methionine,[11] adenine,[12, 13] guanine,[13] xanthine,[13] and hypoxanthine,[13] which might be present in the extracts. It was therefore decided to test the effects of *p*-aminobenzoic acid, methionine, xanthine, hypoxanthine, and adenine on the respiration of yeast and on the respiratory-depressing action of phenylmercuric nitrate. Guanine was not studied because of its extremely low solubility in the respiration medium.

Inasmuch as none of the substances reported in this paper counteracts the respiratory-depressant effects of phenylmercuric nitrate, experiments have been undertaken on the effects of phenylmercuric nitrate on enzyme systems and on the effects of sulfhydryl-containing compounds on phenylmercuric nitrate activity. These experiments, to be published later, indicate that phenylmercuric nitrate can be antagonized by sulfhydryl-containing compounds, and that it depresses several enzyme systems.

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[10]Dubos, R.: *Ann. Rev. Biochem.* **11** (1942): 662.

[11]Harris, J. S., and Kohn, H. I.: *J. Pharmacol.* **73** (1941): 383.

[12]Martin, G. J., and Fisher, C. V.: *J. Biol. Chem.* **144** (1942): 289.

[13]Snell, E. E., and Mitchell, H. K.: *Arch. Biochem.* **1** (1942): 93.

EXPERIMENTAL

All experiments were made by the direct Warburg method at 37.5° C. in a manner similar to that used in earlier studies[3, 14]. A pure strain of *Saccharomyces cerevisiae*, as used in our yeast growth work,[15] was employed. It was washed from agar slants and made up in M/15 potassium dihydrogen phosphate containing 0.02 per cent glucose to such concentration that 1 ml. added to the respirometer flask contained 4 mg. dry weight. The total volume of liquid in the outer well of the flask was 3 ml., this being made up with M/15 potassium dihydrogen phosphate containing 0.02 per cent glucose at pH 4.5. All dilutions of test substances were made with this medium. All determinations were made in duplicate. Readings were recorded at 15-minute intervals for a period of 1 hour.

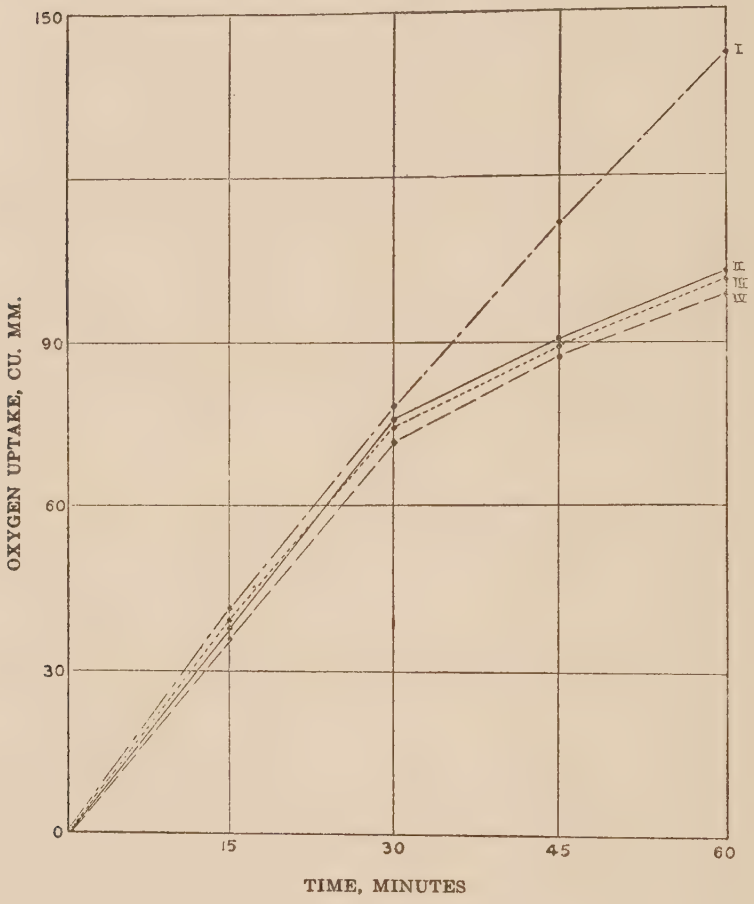
RESULTS AND DISCUSSION

p-Aminobenzoic Acid. To determine the effects of *p*-aminobenzoic acid upon the respiration of the yeast, it was placed in the flask at the beginning of the experiment in various concentrations obtained by adding 1 ml. of the proper dilution (made with M/15 KH_2PO_4) of a 2.2×10^{-2} M stock solution. Concentrations ranging from 7×10^{-7} to 2.2×10^{-4} M had no effect on yeast respiration. Higher concentrations (9×10^{-4} M to 7.3×10^{-3} M) lowered the oxygen uptake of the yeast. At the end of 1 hour the percentage depressions of respiration for the various concentrations were: 9×10^{-4} M, 23 per cent; 1.8×10^{-3} M, 27 per cent; 3.7×10^{-3} M, 42 per cent; 7.3×10^{-3} M, 74 per cent. Depressions were not considered significant unless they were greater than 10 per cent. *p*-Aminobenzoic acid in a concentration of 4.5×10^{-4} M caused a respiratory depression

[14] Cook, E. S., Kreke, C. W., and Nutini, L. G.: *These Studies* **2** (1938): 23; Cook, E. S., and Morgan, Sr. M. N.: *Biochem. J.* **34** (1940): 185.

[15] Cook, E. S., and Cronin, Sr. A. G.: *These Studies* **3** (1941): 205.

FIG. 1. *Effects of para-Aminobenzoic Acid and Phenylmercuric Nitrate on the Respiration of Yeast.*



Curve I. Control. *Curve II.* Phenylmercuric nitrate, final concentration 1.5 x 10^{-5} M (1:100,000), tipped in at 30 min. *Curve III.* *p*-Aminobenzoic acid, final concentration 7.3 x 10^{-5} M (1:100,000), initially present; phenylmercuric nitrate, final concentration 1.5 x 10^{-5} M (1:100,000), tipped in at 30 min. *Curve IV.* *p*-Aminobenzoic acid, final concentration 1.5 x 10^{-5} M, initially present; phenylmercuric nitrate, final concentration 1.5 x 10^{-5} M, tipped in at 30 min.

of 11 per cent at the end of 1 hour and may therefore be regarded as approximately the threshhold concentration above which significant depression occurs.

In studying the possible effects of *p*-aminobenzoic acid on the respiratory-depressing action of phenylmercuric nitrate, we first confirmed our earlier findings[3] that phenylmercuric nitrate (basic) in a concentration of 1.5×10^{-5} M (approximately 1:100,000) depressed the respiration of yeast when it was added either at the beginning of the experiment or 30 minutes later. The effects of *p*-aminobenzoic acid and phenylmercuric nitrate are shown in Figure 1. Curve I represents oxygen consumption by a yeast control and shows that the rate of respiration remains constant over the experimental period of 1 hour. Curve II shows the result of tipping in phenylmercuric nitrate in a final concentration of 1.5×10^{-5} M (1:100,000) at 30 minutes. The depression is evident. In the experiment represented by Curve III, *p*-aminobenzoic acid at a final concentration of 7.3×10^{-5} M (1:100,000), or an initial concentration of 11×10^{-5} M, was present in the flask at the beginning of the experiment, and the phenylmercuric nitrate, final concentration 1.5×10^{-5} M, was tipped in at 30 minutes. Since Curves II and III are superimposable within experimental error, it is evident that the *p*-aminobenzoic acid had no adverse effect on yeast respiration nor did it antagonize the action of the phenylmercuric nitrate when both were present in the same weight concentrations. Curve IV represents the results of a similar experiment except that the *p*-aminobenzoic acid and phenylmercuric nitrate were finally present at equimolar concentrations (1.5×10^{-5} M). This curve, within experimental error, is practically identical with Curves II and III. Repetitions of the experiment gave similar results.

Methionine. Methionine, added in concentrations ranging from 1.3×10^{-5} to 3.3×10^{-3} M, did not significantly affect the respiration over a test period of an hour. Experiments similar

to those conducted with *p*-aminobenzoic acid demonstrated that methionine in concentrations of from 1.66 x 10^{-3} to 1.33 x 10^{-2} M exerted no protective action against 1.5 x 10^{-5} M phenylmercuric nitrate.

Xanthine and Hypoxanthine. Xanthine in concentrations of 1.3 x 10^{-4} and 6.6 x 10^{-5} M had no effect on yeast respiration or on the depressing effect of 1.5 x 10^{-5} M phenylmercuric nitrate. The low solubility of xanthine prevented trial of stronger concentrations. Similarly, hypoxanthine in concentrations from 9.2 x 10^{-4} to 7.4 x 10^{-3} M did not affect yeast respiration, nor did it modify the respiratory depression caused by 1.5 x 10^{-5} M phenylmercuric nitrate.

Adenine. Adenine, as the sulfate, was tested in concentrations from 4.1 x 10^{-4} to 6.6. x 10^{-3} M (calculated as adenine). Some depression of respiration occurred, the amount being dependent upon the concentration of adenine. The greatest depression was 20 per cent at the end of a respiration period of one hour, caused by 6.6 x 10^{-3} M adenine. The presence of adenine in the flask prior to the addition of 1.5 x 10^{-5} M phenylmercuric nitrate did not unexpectedly modify the toxicity of the latter. Respiration in the presence of adenine was lower than that of the control, the degree of depression depending upon the concentration of the adenine, the addition of the phenylmercuric nitrate simply causing an additional lowering of the respiratory rate. The adenine apparently did not offset respiratory depression by the germicide nor did it enhance the depression beyond the additive effects.

SUMMARY

para-Aminobenzoic acid in concentrations above 4.5 x 10^{-4} M depressed the respiration of yeast. In a concentration of 1.5 x 10^{-5} M it had no effect upon the depression of respiration caused by equimolar phenylmercuric nitrate (1:100,000). It

had no effect on oxygen-uptake when present in equal weight with the germicide (1:100,000 or 7.3×10^{-5} M) concentration.

Methionine, in concentrations from 1.3×10^{-5} to 3.3×10^{-3} M did not significantly affect yeast respiration nor did it exert protective action against depression of the rate by phenylmercuric nitrate.

Xanthine, 1.3×10^{-4} to 6.6×10^{-5} M, and hypoxanthine, 9.2×10^{-4} to 7.4×10^{-3} M, did not affect yeast respiration nor the depression of respiration by phenylmercuric nitrate.

Adenine, 4.1×10^{-4} to 6.6×10^{-3} M (added as sulfate) caused slight depression of yeast respiration and had no effect, other than additive, on the depression caused by phenylmercuric nitrate.

Under the experimental conditions described, the effects of none of the above substances would seem to account for the ability of yeast extracts to offset the respiratory-depressing effects of phenylmercuric nitrate on yeast.

Original Manuscript Submitted
May 1, 1944

ACTION OF YEAST EXTRACTS IN STIMULATING ANIMAL TISSUE RESPIRATION

CORNELIUS W. KREKE AND
SISTER MARY ST. AGATHA SUTER, I.H.M.

In previous communications,[1, 2] evidence was presented to show that yeast extracts with respiratory-stimulating action were able to antagonize potassium cyanide and sodium azide depression of respiration but did not have any effect in the presence of amyl alcohol or urethane. The present study was undertaken to determine if the action of yeast extract on respiration was the same for rat skin and liver as for yeast.

EXPERIMENTAL

Warburg respirometers were used, 2 flasks serving as barometric controls, 2 as tissue controls, and 10 as experimental flasks, 2 for each concentration of the poison. Measurements of oxygen-uptake were made at 37.5° C. in Ringer-phosphate glucose solution (R.P.G.) containing 0.02 per cent glucose. Control flasks contained 3 ml. R.P.G. and approximately 20 mg. dry weight of rat skin or liver. The tissues, prepared as described in a previous publication[3] were obtained from 5-month-old female rats of a strain obtained several years ago from Anheuser-Busch. The experimental flasks contained in place of 2 ml. of the R.P.G., 1 ml. of respiratory poison and 1 ml. of yeast extract, both dissolved in R.P.G. to the desired concentration. Two-tenths ml. of N potassium hydroxide was present in the inner well of each flask to absorb carbon dioxide.

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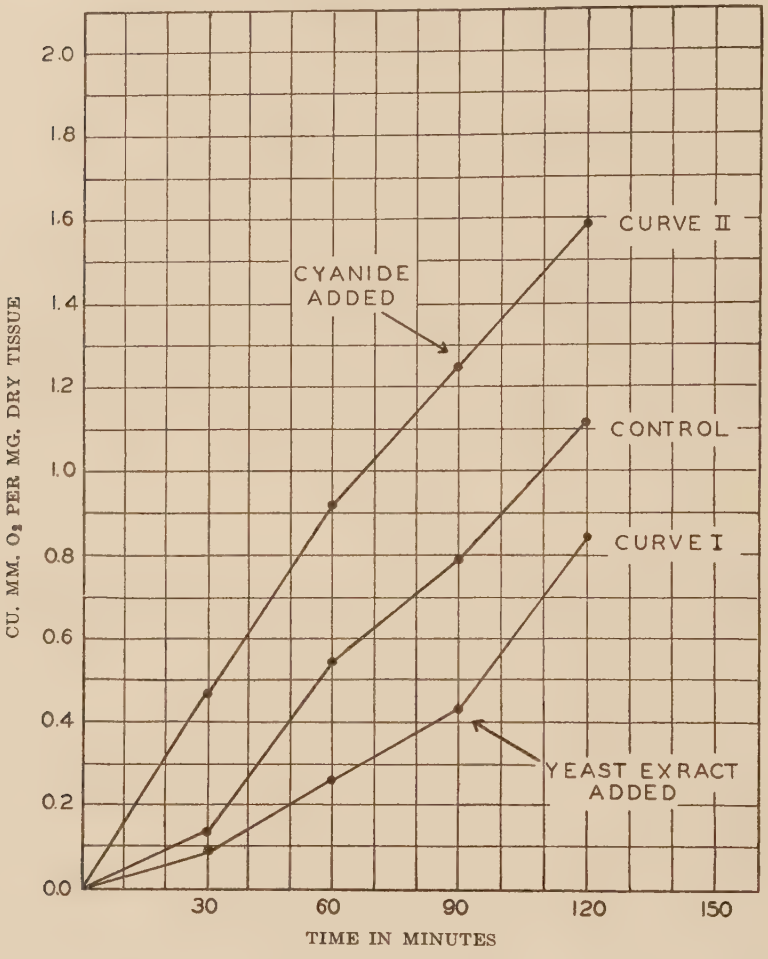
[1] KREKE, C. W., AND SUTER, SR. M. ST. A.: *J. Biol. Chem.* **160** (1945): 105.

[2] KREKE, C. W., BARTLETT, SR. M. D., AND SUTER, SR. M. ST. A.: *Nature* **154** (1944): 268.

[3] COOK, E. S., KREKE, C. W., AND NUTINI, L. G.: *These Studies* **2** (1938): 23.

FIG. 1. *Effect of Yeast Extract (6 mg./ml.) on Cyanide (M/5600) Depression of Skin Respiration.*



Control . . . . . Skin+R.P.G.
Curve I . . . . . Skin+R.P.G.+Cyanide. Extract added at 90 min.
Curve II . . . . . Skin+R.P.G.+Extract. Cyanide added at 90 min.

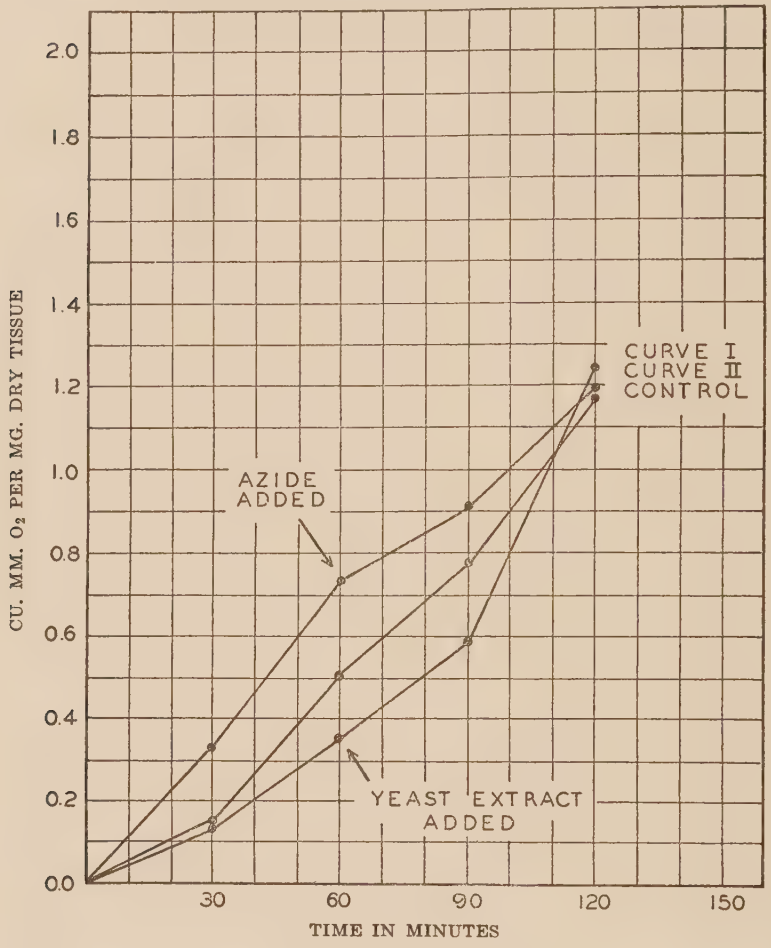
The respiratory poisons used were potassium cyanide, sodium azide, urethane, and amyl alcohol. The final concentrations of cyanide and azide ranged from M/700 to M/60,000, and

those of the urethane and amyl alcohol from M/350 to M/5600. Oxygen consumption was measured at least twice for each dilution of the poison employed. The yeast extract was present in a final concentration of 6 mg./ml.

Two experimental procedures were adopted to determine if the respiratory-stimulating activity of yeast extract could counteract the depressant action of respiratory poisons. In the first, tissue was added to the poison-containing medium and after a respiratory period of 60 or 90 minutes, yeast extract was added from the side arm of the flask; in the second, the order was reversed by adding tissue to the extract-containing medium and 60 and 90 minutes later the respiratory poison was added. In order to maintain uniform conditions for the control and experimental flasks, 1 ml. of R.P.G. was spilled from the side arm of the control tube at the same time that the poison or extract was spilled into the experimental chamber. In some experiments the respiratory rate dropped slightly, apparently as the result of the mechanical operation. Respiration was studied over a two-hour experimental period in each case.

Cyanide. In Figure 1 is shown the effect of yeast extract on cyanide depression of oxygen consumption by rat skin. When the tissue was allowed to respire in the presence of cyanide (M/5600) there was a 45 per cent depression of the rate after 90 minutes (Curve I). When yeast extract was added there was an immediate increase in the rate of respiration above that of the control as shown by the rise in the slope of the curve in the next 30 minutes. In the second experiment (Curve II), respiration of the skin was stimulated approximately 55 per cent by the yeast extract in the initial 90-minute period and when cyanide (M/5600) was added at this time, the respiratory rate remained the same. The yeast extract at once offset completely the depressant effect of cyanide. With

FIG. 2. *Effect of Yeast Extract (6 mg./ml.) on Azide (M/1400) Depression of Skin Respiration.*



Control.....Skin+R.P.G.
Curve I.....Skin+R.P.G.+Azide. Extract added at 60 min.
Curve II.....Skin+R.P.G.+Extract. Azide added at 60 min.

the amount of yeast extract used (6 ml.) the effects of increasing the concentrations of cyanide were offset to less degree.

Azide. The respiratory depressant action of azide (M/1400) on rat skin was less in a 60-minute period than that of cyanide

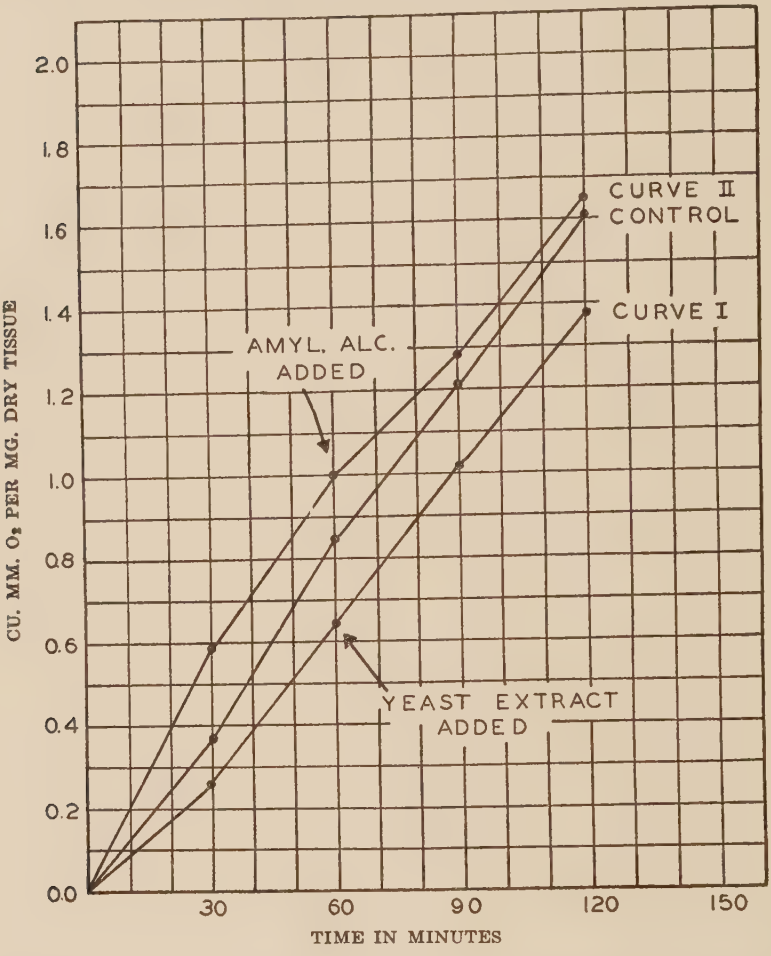
(M/5600) in the same time interval, the depression by azide being approximately 30 per cent (Curve I, Fig. 2). When yeast extract was added there was no change in the rate of respiration in the succeeding 30 minutes, at the end of which time there was a sharp increase as shown by the rise in the slope of Curve I, Figure 2. In the second part of the experiment (Curve II) there was a 25 per cent increase in the oxygen-uptake 60 minutes after tissue was immersed in yeast extract and the medium. When azide was added at this time there was an initial depression of the respiratory rate as shown by the slope of the curve during the next 30 minutes, after which the rate approached that of the normal; in other experiments of the same type the rate returned to normal.

Yeast extract overcame the depressant action of azide on skin respiration only after an initial lag period of 30 minutes, whether the extract acted before or after exposure of the tissue to the azide. The degree of recovery of the respiratory rate depended in some measure upon the lapse of time following addition of the extract or azide.

Amyl Alcohol. Amyl alcohol (M/1400) depressed skin respiration 30 per cent in a 60-minute period (Curve I, Fig. 3). When yeast extract (6 mg./ml.) was added at this time there was no change in the rate of respiration. In the second part of the experiment (Curve II) the extract stimulated the oxygen consumption 20 per cent in the initial 60-minute period. When the amyl alcohol (M/1400) was added at the end of this time, a slight depression in the succeeding 30 minutes was followed by an increase to nearly that of the normal as shown by the slope in Curve II, Figure 3.

In the other experiments with varying concentrations of amyl alcohol in the initial period, the yeast extract (6 mg./ml.) had no effect on the slightly depressed respiratory rate. On

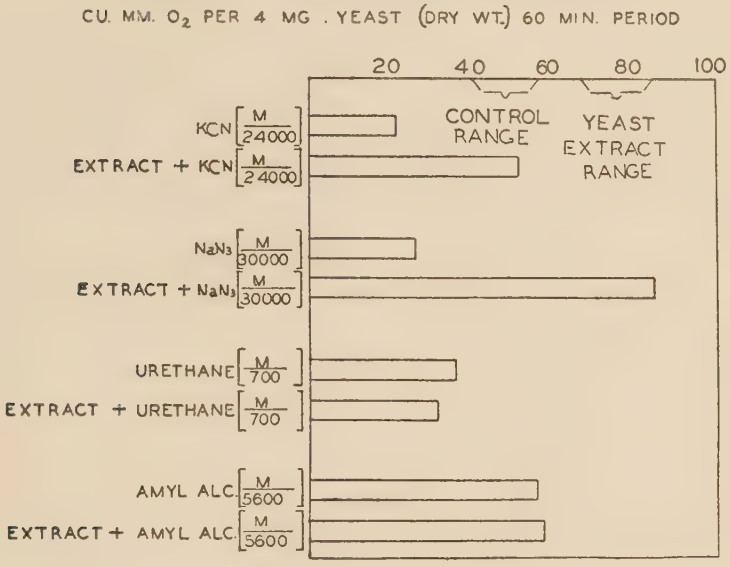
FIG. 3. *Effect of Yeast Extract (6 mg./ml.) on Amyl Alcohol (M/1400) Depression of Skin Respiration.*



Control.....Skin+R.P.G.
Curve I.....Skin+R.P.G.+Alcohol. Extract added at 60 min.
Curve II.....Skin+R.P.G.+Extract. Alcohol added at 60 min.

the other hand, the increased rate resulting from yeast extract in the initial period was but slightly depressed by the action of amyl alcohol.

FIG. 4. *Yeast Respiration. Antagonistic Action of Yeast Extract Against Respiratory Poisons.*



Urethane. Contrary to expectations, urethane, at least in the concentrations used (M/350-M/5600), produced a small stimulation rather than a depression of respiration of the skin. In the presence of urethane, as with amyl alcohol, yeast extract did not stimulate oxygen consumption. This is illustrated in the bargraph of Figure 5.

Liver. Liver slices behaved similarly to skin since the depression of liver respiration by potassium cyanide and sodium azide could be overcome by the addition of the yeast extract. In contrast, however, yeast extract stimulated oxygen consumption by the liver in the presence of urethane and amyl alcohol.

In Figures 4, 5 and 6 is given a summary of the results on oxygen consumption by skin, liver, and yeast, the data for yeast being included for comparison. The units in the bar-

FIG. 5. *Rat Skin Respiration. Antagonistic Action of Yeast Extract Against Respiratory Poisons.*

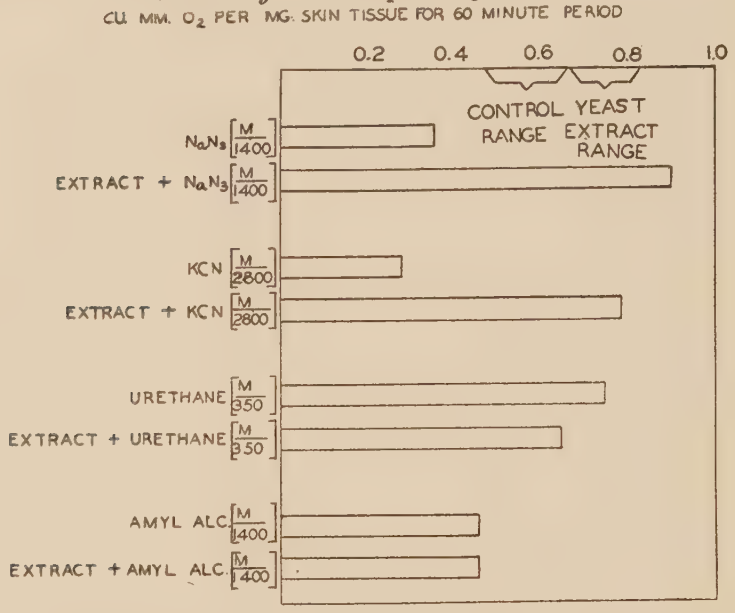
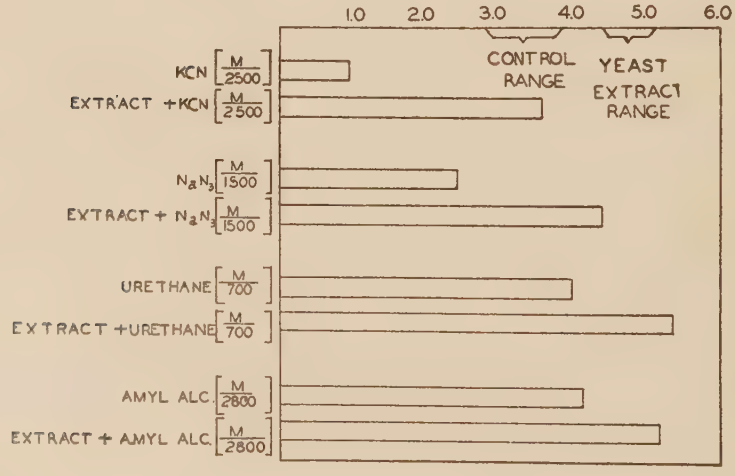


FIG. 6. *Rat Liver Respiration. Antagonistic Action of Yeast Extract Against Respiratory Poisons.*



graphs are in total cu. mm. of oxygen per mg. dry weight of tissue, and 4 mg. dry weight of yeast. The graphs show clearly that, in all three types of cells, depression of respiration by potassium cyanide and sodium azide could be completely overcome by the yeast extract.

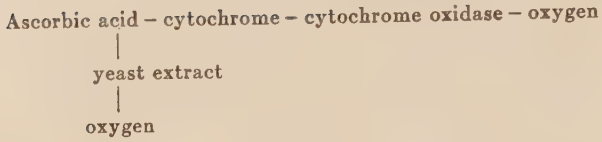
The results with urethane and amyl alcohol are less clear cut since it was found that these substances in the concentrations used did not depress respiration. The yeast extract, however, was not capable of stimulating oxygen-uptake in the presence of these substances except in the case of liver in which there seemed to be an additional oxygen-uptake over and above the increase due to both urethane and amyl alcohol.

DISCUSSION

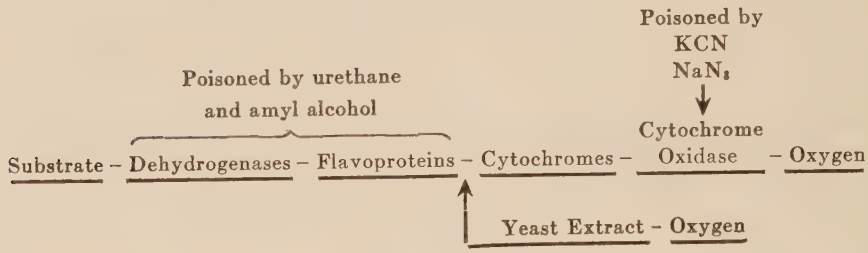
Because it is generally believed that potassium cyanide or sodium azide selectively poison the cytochrome oxidase while amyl alcohol and urethane interfere with the cytochrome-reducing portion of the respiratory chain, these 4 poisons were chosen to study the mechanism of respiratory stimulation by yeast extract. From the evidence presented it appears that the yeast extract is concerned with the cytochrome portion of the chain since potassium cyanide and sodium azide poisoning in the concentrations employed could be completely overcome by yeast extract. The extract had no activity in the presence of urethane and amyl alcohol poisoning, with the exception of liver in which there was some stimulation of oxygen consumption by yeast extract in the presence of the poisons. We do not, however, feel that this stimulation is important since the amyl alcohol and/or urethane stimulated the oxygen-uptake of liver.

It has been shown in our laboratories (unpublished data) that when yeast extract was added to the cytochrome *c*-cytochrome oxidase-ascorbic-acid system of Schneider and

SCHEME I



SCHEME II



Potter[4] the oxidation of ascorbic acid was increased; in fact the cytochrome enzymes were by-passed since the ascorbic acid was oxidized by the extract in the absence of cytochrome *c* and cytochrome oxidase (see Scheme 1). This led us to the view that the yeast extract may contain autoxidizable substances which carry oxygen directly to hydrogen acceptors as shown in Scheme 2.

It is quite possible that other mechanisms exist to explain the antagonism of yeast extract for potassium cyanide and sodium azide poisoning. Some substances in the extract may inactivate the poison while simultaneously others stimulate respiration. We do not feel that it is necessary to assume a separate mechanism for these two actions since we have found, (unpublished data) that there is a relationship between the ability of the extract to stimulate respiration and to inactivate poisons. Previous evidence has shown that active yeast extracts accelerate the action of peroxidase[5] in oxidizing sub-

[4]SCHNEIDER, W. C., AND POTTER, V. R.: *J. Biol. Chem.* **149** (1943): 217.

[5]KREKE, C. W., AND BARTLETT, SR. M. D.: *These Studies* **4** (1945): 1; 15.

strates with hydrogen peroxide and of the enzyme catalase[2, 6] in splitting hydrogen peroxide. Yeast extract can overcome potassium cyanide and sodium azide poisoning of these enzymes. The mode of action of the yeast extract in accelerating the iron-containing enzymes appears to differ from that in the stimulation of respiration, for the yeast extract of itself has no peroxidase or catalase activity.

Since yeast extracts overcome depression of skin[7] and yeast[8] respiration by phenylmercuric nitrate, it seems likely that the germicide affects the cytochrome-oxidase portion of the respiratory chain. On the other hand, the depression caused by certain local anesthetics cannot be so overcome[9] and it is therefore possible that these anesthetics, in common with narcotics, affect the dehydrogenating part of the respiratory chain of enzymes. These possibilities are being investigated.

SUMMARY

Evidence has been presented to show that yeast extracts exert an antagonistic action against respiratory poisons such as potassium cyanide and sodium azide. No such antagonistic action could be shown against amyl alcohol or ethyl urethane. It has been concluded from experimental evidence that the respiratory-stimulating action of yeast extracts is concerned with the cytochrome portion of the respiratory chain.

Original Manuscript Submitted
December 27, 1944

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[6] KREKE, C. W., BARTLETT, SR. M. D., AND SMALT, SR. M. A.: *J. Biol. Chem.* **158** (1945): 469.

[7] COOK, E. S., KREKE, C. W., EILERT, SR. M. R., AND SAWYER, M. A.: *Proc. Soc. Exptl. Biol. Med.* **50** (1942): 210.

[8] COOK, E. S., AND KREKE, C. W.: *Proc. Soc. Exptl. Biol. Med.* **53** (1943): 222.

[9] COOK, E. S., AND MCDEVITT, SR. M. OF L.: *These Studies* **4** (1945): 107.

EFFECT OF VARIOUS TISSUE EXTRACTS ON THE GROWTH OF COLON-TYPHOID-DYSENTERY ORGANISMS

LEO G. NUTINI, SISTER MARY EDWARD LEO HENNESSEY, S.S.J., AND SISTER EVA MARIA LYNCH, S.S.J.

In several previous papers,[1, 2] we have reported the ability of spleen extract to stimulate the growth of certain microorganisms, and to inhibit or completely kill certain others. Among those organisms exhibiting increased growth when subjected to the action of this extract were a representative number of the colon-typhoid-dysentery group. The present work, concerned solely with this group, specifically *Escherichia coli*, *E. coli communior*, *Eberthella typhosa*, *Bacillus dysenteriae* (*Shigella*), *Salmonella enteritidis* and *Bacillus paratyphosus A*, was undertaken with the express purpose of determining whether this stimulatory material resided only in the spleen or could be extracted from other organs as well, namely heart, brain, kidney, and liver.

We have not attempted in this short article to review the extensive literature on plant and animal tissues as sources of growth or "accessory" food factors and growth stimulants for bacteria. For information on this subject the reader is referred to the work of Koser, *et al.*,[3] and the excellent summary of Peskett[4]. It is, however, apparent in checking this literature that little is known regarding the mechanism of action of

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[1] NUTINI, L. G., AND KREKE, C. W.: *J. Bact.* **44** (1942): 661.

[2] NUTINI, L. G., KREKE, C. W., AND SCHROEDER, SR. M. P.: *J. Bact.* **50** (1945): 177.

[3] KOSER, S. A., SAUNDERS, F., FINKLE, I. I., AND SPOELSTRA, R. C.: *J. Infect. Dis.* **58** (1936): 121; KOSER, S. A., AND SAUNDERS, F.: *Bact. Rev.* **2** (1938): 99.

[4] PESKETT, G. L.: *Biol. Rev.* **8** (1933): 1.

most of these substances. It is further apparent that none of the materials described possess the diversity of action on bacteria that has been exhibited by splenic extract.

EXPERIMENTAL

Extracts of beef spleen, heart, brain, kidney, and liver were prepared in a manner identical to that previously described[1]. After preparation of the extracts each was tested a minimum of 4 times for its effect on the following six organisms: *Escherichia coli*, *E. coli communior*, *Salmonella enteritidis*, *Eberthella typhosus*, *Bacillus paratyphosus A* and *Bacillus dysenteriae* (*Shigella*). A series of three Petri dishes, one serving as a control, the other two as experimental, was prepared for each organism. All three dishes contained 20 cc. of nutrient agar, the experimental differing from the control in that one contained 0.5 per cent, the other 1.0 per cent dry weight of the extract to be tested. Each dish was inoculated with 0.1 ml. of a saline-diluted (1:1,000,000) 24-hour culture of the organism and swirled several times to insure an equal distribution of the colonies for facilitating the counting with the Wolffhuegel plate counter after the dishes were incubated at 37° C. for 48 hours.

From these experiments it was clearly evident that the stimulatory material is not confined to spleen alone, but is found in other organs as well. Table I, recording the stimulation of the 6 organisms by the different extracts in terms of percentage, is based directly on total plate counts. It will be noted from these results that heart and kidney extracts produce a greater percentage stimulation of growth than brain, spleen, and liver extracts, the latter three being approximately equal in their stimulative power. It is to be noted, moreover, that one of the extracts (liver) undergoes a change in its activity if it is stored in the refrigerator. The extract when used before storage showed stimulatory activity, while after storage there

TABLE I. *Percentage Growth Stimulation of Colon-Typhoid-Dysentery Organisms Subjected to the Action of Various Tissue Extracts.*

Organism	Growth Response to 0.5 and 1% Extracts of											
	Heart		Kidney		Brain		Spleen		Liver Before Storage		Liver After Storage	
	0.5%	1.0%	0.5%	1.0%	0.5%	1.0%	0.5%	1.0%	0.5%	1.0%	0.5%	1.0%
E.coli	171	466	8.6	115	18	59.3	15.7	21.3	23.6	23.9	21.9	10.0
E.coli communior	228	425	54.0	116	29	52.0	34.0	43.6	2.6	2.6	−21.9	−53.6
S.enteriditis	225	440	48.0	87	36	70.0	18.0	38.0	58.0	49.0	1.6	− 2.4
E.typhosus	325	606	45.0	124	47	97.0	44.1	76.0	31.3	12.1	−17.4	−38.0
B.paratyphosus A	240	508	221.0	175	46	74.0	24.0	92.0	2.5	4.8	− 9.8	−23.7
B.dysenteriae	157	386	94.0	151	48	103.0	49.6	39.7	58.0	49.0	−47.1	−63.7

was a reduction, in some individual experiments, as high as 100 per cent.

Work is now in progress to determine the reason for the change in the liver extract during storage. Investigations on the effects of the stored extract on *B. dysenteriae* (*Shigella*) infections in mice have also been undertaken.

SUMMARY

1. The action of heart, kidney, brain, and liver extracts on the colon-typhoid-dysentery group of organisms has been found to resemble that of spleen in that they stimulate the growth of these organisms. Extracts of the heart and kidney are much more active than those of the brain and liver.

2. Liver extract undergoes a change upon storage in the refrigerator and produces an inhibition in growth. The cause responsible for this change is under investigation.

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April 6, 1945

EFFECT OF THE FILTRATE OF ULTRAVIOLET IRRADIATED *STICHOCOCCUS BACILLARIS* NAEGELI UPON THE CHLOROPHYLL AND CARBOHYDRATE[1] CONTENT OF ALGAE[1]

SISTER MARY REDEMPTA EILERT, C.S.J., AND
SISTER CRESCENTIA GIERSCH, C.S.J.[2]

In earlier papers from this laboratory it was shown that the cell-free filtrate from suspensions of *Hormidium floccidum*[3] and *Stichococcus bacillaris*[4] which had been treated with ultraviolet light contained a substance or substances which when added to an algal suspension stimulated cellular proliferation. Other investigators[5] have reported the presence of similar substances in yeast and animal tissues which have been irradiated.

Questions concerning the nature of this growth-promoting substance, and the mechanism involved in its action on the algal cells have naturally arisen. The present study of the effect of the irradiated cell-free filtrate upon the carbohydrate and chlorophyll content of the unicellar alga, *Stichococcus bacillaris* Naegeli was suggested as offering a possible explanation for the increased growth produced by the irradiated filtrate. If it could be shown that the amount of reducible carbohydrates per unit weight of plant tissue was greater in cells grown in the presence of the growth factor, a reasonable

[1]Assistance in the experimental work was given by Miss Geraldine Beisner.

[2]From the Marymount College Unit of the Institutum Divi Thomae, Salina, Kansas.

[3]GIERSCH, SR. C., AND COOK, E. S.: *Nature* **148** (1941): 754.

[4]GIERSCH, SR. C., *These Studies* **4** (1945): 51.

[5]FARDON, J. C., CARROLL, SR. M. J., AND RUDDY, SR. M. V.: *These Studies* **1** (1937): 17; SPERTI, G. S., LOOFBOUROW, J. R., AND LANE, SR. M. M.: *Science* **86** (1937): 611; LOOFBOUROW, J. R., DWYER, SR. C. M., AND LANE, SR. M. M.: *Biochem. J.* **34** (1940): 432.

conclusion would be that, by stimulating carbohydrate production, these cells were furnished with more nutrient matter than the control cells. Similar reasoning prompted the investigation on chlorophyll content of the algae.

EXPERIMENTAL

Preparation of the irradiated filtrate. Stock cultures of *Stichococcus bacillaris* were grown on Detmer's glucose-agar medium for about ten days. The algal suspension was prepared by washing the growth from twelve sixteen-ounce bottles using one liter of Detmer's ⅓. The suspension was divided into equal parts and placed in open vessels. One portion was treated with ultraviolet light (Sperti Mercolite bulb) at a distance of 15 cm. for a two-hour period, and both suspensions were stirred mechanically with the same motor during irradiation. At the end of the irradiation period, the algal suspensions were filtered through Berkefeld filters. The irradiated filtrate was designated as I and the non-irradiated filtrate as N.

Procedure for growing algae. Earlier tests on the irradiated filtrate had shown that the growth factor withstood autoclaving. In determining the effect of the filtrates on the carbohydrate and chlorophyll content of algae the following procedure was used: 50 ml. of I was added to each of three flasks containing 150 ml. of basal medium, and the flasks autoclaved for 15 minutes at 15 pounds pressure. One hundred ml. of an algal suspension was added aseptically to each flask after cooling. The control flasks were prepared in a similar manner using 50 ml. of N. The flasks were placed in a cool room with a northern exposure for two weeks. At the end of the growing period, the suspensions were filtered and the carbohydrate and chlorophyll content of the algae were determined. For the carbohydrate determination, the algae were washed with distilled water until a negative Molisch test was obtained, and then they were scraped from the candles

and weighed. Both dry and wet weight determinations were made in all experiments.

Carbohydrate and chlorophyll analyses. The following procedure was adopted in determining total sugars, starch, and hemicelluloses in the algae which were removed from the Berkefeld candles. Approximately 500 mg. of the wet plant tissue were weighed and then transferred quantitatively to a small Erlenmeyer flask and refluxed with approximately 2.5 per cent hydrochloric acid for two and one-half hours. The hydrolyzed plant extract was neutralized with anhydrous sodium carbonate, clarified with neutral lead acetate and de-leaded with dibasic sodium phosphate. After the clarified solution was made up to a definite volume and filtered, it was analyzed for reducing sugars using Forbee's photocolorimetric ferricyanide[6] method. Color decrease was measured quantitatively with a Cenco photelometer (Industrial Type B) using a blue filter. The glucose content of the plant extract was read from a standard glucose curve.

Chlorophyll extractions were made on weighed amounts of the wet plant tissue following the method of Schertz[7]. The extract was made up to a definite volume and the chlorophyll content determined by means of a Cenco photelometer (Industrial Type B). A standard was prepared using 5x chlorophyll purchased from American Chlorophyll, Inc.

DISCUSSION AND CONCLUSIONS

Table I summarizes the data obtained in this investigation on the carbohydrate content of the alga. I_1 represents extracts obtained from the alga immediately after irradiation (N_1, control). I_2 and N_2 are extracts obtained from algae which had been grown in the presence of the irradiated and non-

[6] Morell, S. A.: *Ind. Eng. Chem., Anal. Ed.* **13** (1941): 249.

[7] Schertz, F. M.: *Plant Physiol.* **3** (1928): 211.

TABLE I. *Carbohydrate Content of Stichococcus Bacillaris Grown in Presence of 50 ml. of Plant Extract.*

Sample	Dry wt. of Alga	Transmission	Glucose equiv.	Dilution	Glucose		Difference I_1-N_1	Difference I_2-N_2
	mg.	%	*mg./ml.*		*mg.*	%		
I_1*	195.5	50.5	0.48	orig.	48.0	24.6		
N_1*	197.2	49.5	0.47	orig.	47.0	23.8	+0.8	
I_2	183.4	40.0	0.37	1:1	37.0	20.2		
N_2	191.6	43.5	0.41	1:1	40.0	21.4		−1.2
I_1	233.7	70.5	0.60	3:2H_2O	50.0	21.5		
N_1	181.0	52.0	0.49	3:2	40.8	22.5	−1.0	
I_2	234.8	50.0	0.47	1:1	47.0	20.0		
N_2	206.8	44.5	0.42	1:1	42.0	20.4		−0.4
I_1	139.8	41.0	0.38	1:1	38.0	27.2		
N_1	125.3	37.5	0.33	1:1	33.0	26.3	+0.9	
I_2	78.2	37.0	0.32	orig.	16.0	20.5		
N_2	109.2	45.0	0.42	orig.	21.0	19.2		+1.3
I_1	114.4	63.5	0.56	orig.	28.0	24.5		
N_1	108.2	58.5	0.53	orig.	26.5	24.5	0.0	
I_2	107.7	44.5	0.42	orig.	21.0	19.5		
N_2	137.1	55.0	0.51	orig.	25.5	18.6		+0.9
I_1	88.0	51.5	0.49	orig.	24.5	27.8		
N_1	86.0	53.5	0.53	orig.	25.0	29.1	−1.3	
I_2	158.0	34.5	0.27	2:3H_2O	33.8	21.4		
N_2	135.8	38.5	0.34	3:2H_2O	28.3	20.8		+0.6
I_1	109.2	72.0	0.60	orig.	30.0	27.5		
N_1	106.4	66.0	0.57	orig.	28.5	26.8	+0.7	
I_2	183.0	36.5	0.31	2:3H_2O	38.8	21.2		
N_2	153.6	34.0	0.26	2:3H_2O	32.5	21.2		0.0
					Average		+.02	+0.2

I_1 Algae analyzed immediately after irradiation.

I_2 Algae treated with irradiated filtrate for 2 weeks before being analyzed.

N_1 Control for I_1. N_2 Control for I_2.

*Volume of plant extract used, 100 ml.

TABLE II. *Chlorophyll Content of Stichococcus Bacillaris.*

Sample	Dry wt. Alga	Dilution	Transmission	Chlorophyll per liter	Chlorophyll per dilution	Chlorophyll per gm. dry wt.	Difference I_2-N_2
	mg.	*ml.*	%	*mg.*	*mg.*	*mg.*	
1. I_2...	296.1	200	30.5	36.5	7.3	24.6	
N_2...	338.8	200	30.0	36.9	7.4	21.8	+2.8
2. I_2...	425.2	400	40.5	27.9	11.2	26.3	
N_2...	466.9	400	42.5	26.8	11.0	23.6	+2.7
3. I_2...	406.4	400	38.5	29.2	11.5	28.2	
N_2...	425.7	400	36.5	30.9	12.3	28.8	−0.6
4. I_2...	158.0	50	35.5	31.9	1.6	10.1	
N_2...	191.1	50	31.0	35.8	1.8	9.4	+0.7
5. I_2...	169.5	50	41.0	28.6	1.4	8.3	
N_2...	170.1	50	38.5	29.2	1.5	8.8	−0.5
6. I_2...	519.5	200	37.5	30.0	6.0	11.5	
N_2...	440.0	200	44.5	25.7	5.7	12.9	−1.4
7. I_2...	448.7	200	42.5	26.8	5.4	11.1	
N_2...	516.6	200	30.0	36.9	7.3	14.1	−3.0
8. I_2...	307.2	100	41.5	27.2	2.7	8.7	
N_2...	240.2	100	33.5	33.8	3.4	9.9	−1.2
9. I_2...	214.3	50	40.5	27.9	1.4	6.7	
N_2...	207.1	50	46.5	24.2	1.2	5.7	+1.0
10. I_2...	376.8	100	32.5	34.7	3.4	9.1	
N_2...	610.8	100	26.5	40.6	4.1	7.1	+2.0
11. I_2...	224.2	50	35.0	32.4	1.6	7.1	
N_2...	269.5	50	30.0	36.9	1.9	7.1	0.0

irradiated filtrates for a period of two weeks. A study of this table reveals the fact that there is no significant difference in the percentage of glucose found in the algae grown in the presence of the irradiated and non-irradiated filtrates. The total glucose content of algae analyzed immediately after irradiation is larger than that of those treated with the irradiated and non-irradiated filtrate. This can be explained by the fact that the algae used in preparing the former extracts, were grown on a glucose-containing medium, whereas the latter were grown on a glucose-free medium.

In a similar manner, Table II gives a summary of the data obtained from the chlorophyll determinations. Due to variations in light and temperature conditions the amount of chlorophyll differed in separate experiments. However, there is no striking difference in the chlorophyll content of the algae treated with the ultraviolet irradiated filtrate.

The data which have been presented seem to indicate that the cell proliferation observed when the cell-free irradiated filtrate is added to a suspension of *Stichococcus bacillaris* does not affect the percentage of carbohydrate or chlorophyll in the algal cells.

Original Manuscript Submitted
May 1, 1945

DEPRESSION OF TISSUE AND YEAST RESPIRATION BY LOCAL ANESTHETICS

ELTON S. COOK AND
SISTER MARY OF LOURDES McDEVITT, I.H.M.

As part of a research program on the effects of drugs on the respiration of tissues and yeast, we have investigated the ability of several local anesthetics to depress the respiration of rat liver and of yeast, and the effects of a yeast extract on this depression. It had been found previously that the respiratory depression caused by phenylmercuric nitrate,[1, 2] cyanide, and azide[3, 4] could be offset, within limits, by a yeast extract but not the depression caused by urethane and amyl alcohol[3, 4]. It had also been observed that the corneal anesthetic activity of cocaine was antagonized by yeast extracts[5].

MATERIALS AND METHODS

The following anesthetics were used and all were purchased on the open market with the exception of diothane, which was kindly furnished by Dr. R. S. Shelton of the Wm. S. Merrell Co.: cocaine hydrochloride, procaine hydrochloride, γ-(2-methylpiperidino)-propylbenzoate hydrochloride (metycaine hydrochloride), piperidinopropanedioldiphenylurethane hydrochloride (diothane hydrochloride), and 2-butoxycinchoninic acid-4-diethylethylenediamide hydrochloride (nupercaine

[1]COOK, E. S., KREKE, C. W., EILERT, SR. M. R., AND SAWYER, M. A.: *Proc. Soc. Exptl. Biol. Med.* **50** (1942): 210.

[2]COOK, E. S., AND KREKE, C. W.: *Proc. Soc. Exptl. Biol. Med.* **53** (1943): 222.

[3]KREKE, C. W., BARTLETT, SR. M. D., AND SUTER, SR. M. ST. A.: *Nature* **154** (1944): 268.

[4]KREKE, C. W., AND SUTER, SR. M. ST. A.: *J. Biol. Chem.* **160** (1945): 105.

[5]COOK, E. S.: *Proc. Soc. Exptl. Biol. Med.* **54** (1943): 203.

hydrochloride). An aqueous-alcoholic yeast extract purified by charcoal treatment[6] was used. Diothane and nupercaine were unsuitable for extensive investigation on liver respiration because of their low solubility and the ease of precipitation of free base at the pH of 7.2. Diothane at 0.0037M and nupercaine at 0.0032M were the highest concentrations obtainable with out precipitation of free base.

The respiration determinations were made by the Warburg method, using procedures similar to those previously described[7]. For the yeast experiments a pure strain of *Saccharomyces cerevisiae* was washed from 72-hour culture slants on Sabouraud's medium. The yeast was made up to such concentration in M/15 potassium dihydrogen phosphate containing 0.02 per cent glucose at pH 4.5 that each respirometer flask contained 4 mg. dry weight. Slices of liver from albino rats 5 to 6 months old, were suspended in Ringer-phosphate solution containing 0.2 per cent glucose at pH 7.2. The temperature was 37.5° C. for tissues and 30° C. for yeast. All determinations were made in duplicate. The anesthetics were prepared in appropriate concentrations and at the requisite pH in the proper medium so that 1 ml. could be tipped into the experimental flask after establishment of the control respiration rate, usually within 20 or 30 minutes. In order to ensure that the respiration rate was constant over the entire experimental period (60 minutes) a separate control was always run.

RESULTS

Effect of Anesthetics on Respiration of Yeast

Diothane. Diothane (0.0115M) caused cessation of respiration of yeast in 10 minutes, and had killed 100 per cent of the

[6] Cook, E. S., and Walter, E. M.: *These Studies* **2** (1939): 189.

[7] Cook, E. S., Kreke, C. W., and Nutini, L. G.: *These Studies* **2** (1938): 23; Cook, E. S., and Morgan, Sr. M. N.: *Biochem. J.* **34** (1940): 185.

cells at the end of the 60-minute experimental period, as shown by methylene blue staining. A concentration reduced by half (0.0058M) of the anesthetic killed approximately 50 per cent of the yeast cells and produced a proportionately smaller respiratory depression.

Nupercaine. Nupercaine (0.016 and 0.033M) killed all of the yeast cells.

Procaine. Procaine (0.125 to 0.5M), in keeping with its low toxicity, caused no depression of yeast respiration.

Cocaine. Cocaine (0.33M) produced apparent complete depression of yeast respiration within 20 minutes but had not killed the cells within 40 minutes after the addition as shown by methylene blue staining.

Metycaine. Metycaine (0.083M) was but slightly toxic for respiration of yeast while double the concentration was definitely toxic. Concentrations of 0.33 and 0.5M caused nearly complete cessation of respiration within 10 to 20 minutes but did not cause killing within 40 minutes as revealed by methylene blue staining.

Effect of Yeast Extract in Counteracting the Toxic Actions of Anesthetic Drugs on Yeast Cells

Yeast extract (10 mg/ml.) did not increase the depressed respiratory rate of yeast cells exposed to diothane (0.0115M) for 20 minutes. When the yeast cells were exposed to the extract prior to the addition of diothane, respiration depression was lessened.

Yeast extract could neither prevent nor counteract the lethal effect of nupercaine (0.016 and 0.033M) on yeast cells.

The complete depression of respiration by 0.33M cocaine at the end of 20 minutes was unaffected by the addition of 10 mg./ml. of yeast extract. Exposure of the yeast cells to the yeast extract for 20 minutes prior to addition of the cocaine

slowed the decrease of respiration, as shown by the fact that at the end of 60 minutes, 50 cu. mm. of oxygen had been consumed after the cocaine (0.33M) had been tipped in, while 23 cu. mm. had been used by the untreated yeast cells. None of the extract-treated cells were killed by the cocaine. A yeast extract (10 mg./ml.) added at the end of a 20-minute exposure of yeast cells to metycaine (0.33 and 0.5M) did not counteract the nearly complete cessation of respiration. Preliminary exposure of the yeast cells to yeast extract increased the oxygen consumption for the 20-minute interval prior to the introduction of metycaine (0.33 and 0.5M) and slowed the rate of decline of respiration, but did not completely prevent it.

Effect of Anesthetics on Respiration of Liver

Diothane. Diothane (0.0037M) had no effect on the oxygen consumption of liver cells during the first 10 minutes after its addition, after which the rate was lowered for 20 minutes and then rose until, at the end of the 60-minute interval, it was nearly as great as that of the control tissue, although the total oxygen consumption in the presence of diothane was 28 per cent less than in the normal tissue.

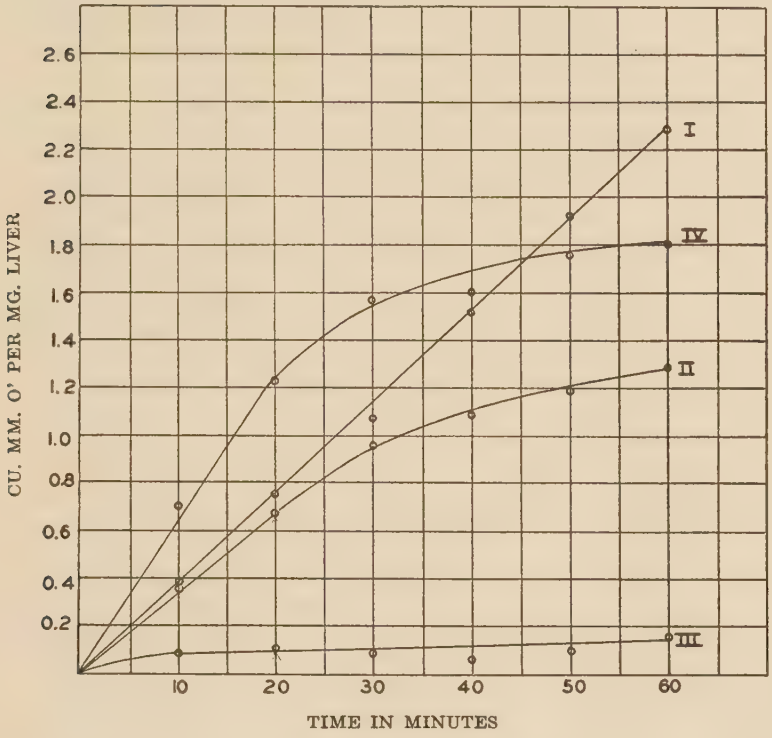
Nupercaine. Nupercaine (0.0032M) produced at most only a temporary depression of respiration which disappeared during the last 20 minutes of the 60-minute experimental period.

Procaine. Procaine in a concentration of 0.5M produced within 30 minutes a 13 per cent depression of oxygen consumption by liver cells.

Cocaine. Cocaine in a concentration of 0.083M caused no depression of liver respiration, while concentrations of 0.167 and 0.33M produced a temporary depression within 20 minutes after its addition, following which the rate returned to normal.

Metycaine. Metycaine in concentrations of 0.167, 0.33 and 0.5M, depressed liver respiration, all concentrations giving nearly identical depressions (32, 33 and 39 per cent, respec-

FIG. 1. *Effects of Metycaine and Yeast Extract on Respiration of Rat Liver.*



Curve I. Respiration of control liver. *Curve II.* Addition of metycaine (0.5M) at 20 minutes. *Curve III.* Metycaine (0.5M) present from beginning, yeast extract (10 mg./ml.) added at 20 minutes. *Curve IV.* Yeast extract (10 mg./ml.) present from beginning, metycaine (0.5M) added at 20 minutes.

tively, in one experiment; that produced by 0.5M in another experiment being shown graphically in Curve II of Fig. 1).

Effect of Yeast Extract in Counteracting the Toxic Actions of Anesthetic Drugs on Liver Cells

Yeast extract in a concentration of 10 mg./ml. when added 20 minutes later than 0.5M metycaine did not overcome the depression of oxygen consumption (Curve III, Fig. 1). Pre-

liminary exposure of the liver to yeast extract produced an increase in oxygen consumption during the first 20 minutes but it did not prevent a depression of respiration when metycaine was added at this time (Curve IV, Fig. 1).

To determine the reversibility of the depression of oxygen uptake in liver cells by metycaine, liver samples were divided into 4 portions. One was incubated in Ringer-phosphate-glucose for 15 minutes at 37.5° C. before placing it in the flask. A second was treated similarly but yeast extract (final concentration 10 mg/ml.) was tipped in after 20 minutes of respiration. The third was incubated with 0.5M metycaine for 15 minutes and rinsed with several portions of Ringer-phosphate-glucose before placing in the flask. The fourth was treated similarly, but yeast extract was tipped in after 20 minutes of respiration.

The liver incubated with Ringer-phosphate-glucose behaved normally and responded to yeast extract stimulation. The liver incubated with metycaine showed an initially depressed rate of respiration which recovered during the experimental period until it was normal or nearly so at the end of 60 minutes; it also responded to yeast extract. Under these conditions metycaine depression of respiration of liver cells is reversible.

DISCUSSION

It is evident that local anesthetics in adequate concentrations generally depress the respiration of yeast and rat liver. Anesthetics, such as diothane and nupercaine, which readily precipitate as free bases are unsuitable for use in the study of tissues at pH 7.2. These anesthetics manifest their relatively high toxicity by killing yeast (methylene blue staining). Procaine, of low toxicity, did not exhibit such toxic effects nor was it active as a respiratory depressant. Metycaine, of

intermediate toxicity, exhibited reversible respiratory depressant effects. Under conditions of the experiments, cocaine and metycaine caused apparent cessation of respiration without killing the yeast. It is possible that sufficiently long exposure to respiratory-depressant concentrations of the anesthetics of intermediate toxicity might cause death. Yeast extracts were ineffective in offsetting respiratory depression by the action of so-called local anesthetics, although the extract, present prior to anesthetic addition, did afford some temporary protection which was more marked with the less toxic anesthetics and which was exhibited more readily with yeast than with liver.

Yeast extracts protect against the depression produced by cyanide and azide, but not against that caused by urethane and amyl alcohol[3, 4]. They increase the activity of catalase[8] and peroxidase[9]. Unpublished experiments show that yeast extracts will bypass the cytochrome-cytochrome oxidase oxidation of ascorbic acid but that they are ineffective with succinic dehydrogenase. These facts suggest that the yeast extracts are effective at the oxidase end of the respiratory chain and may act by the presence of autoxidizable substances which carry oxygen directly to hydrogen acceptors. Consonant with these suggestions is the lack of influence of yeast extracts on respiratory depression by local anesthetics which, like that due to narcotics, may be presumed to involve the dehydrogenases, or some link nearer to the oxidase[10]. These matters are under investigation and any conclusive pronouncement as to mechanism must await completion of these further studies.

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[8]KREKE, C. W., BARTLETT, SR. M. D., AND SMALT, SR. M. A.: *J. Biol. Chem.* **158** (1945): 469.

[9]KREKE, C. W., AND BARTLETT, SR. M. D.: *These Studies* **4** (1945): 1; 15.

[10]MICHAELIS, M., AND QUASTEL, J. H.: *Biochem. J.* **35** (1941): 518; GREVILLE, G. D.: *Nature* **148** (1941): 320.

SUMMARY

Local anesthetics (cocaine, diothane, nupercaine, procaine, and metycaine) depress the respiration of yeast and of rat liver in a manner roughly proportional to the toxicity of the anesthetics. The most toxic (diothane and nupercaine) kill yeast as demonstrated by methylene blue staining. Yeast extracts do not offset the toxic effects on respiration although they may afford some temporary protection if present before the anesthetic is added.

EFFECT OF TISSUE EXTRACTS ON GROWTH OF AVIRULENT AND VIRULENT TUBERCLE BACILLI *IN VITRO*

LEO G. NUTINI, GIRARD W. THOMAS, AND JOSEPH SMOLAR, C.PP.S.

RECENT investigations in these laboratories[1, 2, 3] have shown that deproteinized extracts prepared from spleen of human and animal origin exert an inhibitory effect on the growth of certain organisms *in vitro* and enhance the development of others. Previous experiments showing that the growth of a virulent strain of the tubercle bacillus was inhibited by low concentrations of spleen extract suggested a more thorough investigation of the effect of splenic as well as other organ extracts—brain, kidney, heart, and liver—on the growth of this organism.

Observations that tissue extracts affect the growth of the tubercle bacillus have been made by a number of investigators. Arloing and his co-workers,[4] testing the effect of non-proteinized glycerin extracts on the growth of the tubercle bacillus, found that extracts of kidney, liver, and spleen from normal guinea pigs retarded growth and that lung extracts enhanced it. Sarnowiec,[5] employing glycerin extracts of tissues obtained from healthy and tuberculous guinea pigs and from rabbits, confirmed this but found no differences between the activity

[1] NUTINI, L. G., AND KREKE, C. W.: *J. Bact.* **44** (1942): 661.

[2] NUTINI, L. G., AND LYNCH, SR. E. M.: *Nature* **156** (1945): 419.

[3] NUTINI, L. G., KREKE, C. W., AND SCHROEDER, SR. M. P.: *J. Bact.* **50** (1945): 177.

[4] ARLOING, F., THEVENAT, L., AND VIALLIER, J.: *Compt. rend. soc. biol.* **124** (1937): 164.

[5] SARNOWIEC, W.: *Z. Tuberk.* **82** (1939): 101.

of tissues from infected and non-infected animals. Hirshberg and Arnold,[6] using human tissue juices obtained by compression, were able to demonstrate that the heated and unheated juices of brain, heart, pancreas, liver, and kidney contained substances inhibitory for the organism. They postulated that the degree of inhibition of the individual juices is comparable to the resistance of that tissue against tuberculosis. The present investigations differ from the foregoing chiefly by nature of the preparation of the extracts.

EXPERIMENTAL

I.

All organ extracts (spleen, brain, kidney, heart, and liver) were prepared according to the method described in a previous publication[1] with the exception that distilled water instead of Drew's solution was added to the tissue pulp prior to freezing and thawing.

In the early experiments, conducted on a non-quantitative basis, two types of media were employed: 1) 6 per cent glycerin agar and 2) Long and Seibert's liquid synthetic medium. Two series of tubes, one containing glycerin agar, the other the synthetic medium, were prepared and to them was added varying concentrations (0.5, 1.0 2.0 and 5.0 per cent) of each of the extracts. The terminal pH of the entire series was 7.2. The tubes containing liquid media were inoculated by floating a small flake of organisms on the surface; the glycerin-agar slants, by seeding with at least three pieces of growth. The incubation period at 37.5° C. for the avirulent strain was 7 days, and for the virulent types 56 days. The tubes containing the latter were sealed with paraffin before incubation.

The test organisms were an avirulent strain of *M. tuberculosis* (American Type Culture Collection No. 607); an H37 strain

[6] Hirschberg, N., and Arnold, L.: *Am. Rev. Tuberc.* 37 (1938): 598.

TABLE I. *Effect of Varying Concentrations of Tissue Extracts on Virulent and Avirulent Tubercle Bacilli.*

Strain	Avirulent M.tuberculosis ATCC 607		Virulent M.tuberculosis H-37—No. 8237		Virulent M.tuberculosis G-2 G-5	
Incubation	7 days		56 days		56 days	
Extract Percent	6% Glycerin Agar	Long and Seibert's Medium	6% Glycerin Agar	Long and Seibert's Medium	Long and Seibert's Medium	
Control..	++++	++++	++++	++++	++++	++++
			Brain			
0.5.....	−	−	++++	+++	+++	+++
1.0.....	−	−	+++	+	++	+
2.0.....	−	−	+	−	+	−
5.0.....	−	−	−	−	−	−
			Kidney			
0.5.....	+++	++++	+++	++	++	++
1.0.....	+++	+++	+	−	−	+
2.0.....	++	+++	−	−	−	−
5.0.....	−	+++	−	−	−	−
			Spleen			
0.5.....	++++	++++	++++	++++	++++	++++
1.0.....	+++	+++	++++	+++	+++	++
2.0.....	+++	−	++++	++	++	++
5.0.....	−	−	++	−	+	−
			Heart			
0.5.....	++++	++++	+++	++	++	++
1.0.....	++++	++++	+	++	++	+
2.0.....	+++	++	−	−	−	−
5.0.....	+++	++	−	−	−	−
			Liver			
0.5.....	++++	++++	++++	++++	++++	++++
1.0.....	++++	++++	++++	++++	++++	++++
2.0.....	++++	++++	++++	++++	++++	++++
5.0.....	++++	++++	−	−	−	−

++++ Good growth; +++ growth; ++ slight growth; + very slight growth; − no growth.

(No. 8237) obtained from the same source; and strain G-2 and G-5 obtained from Dr. Guy P. Youmans of the Northwestern University Medical School. All of the organisms were grown on glycerin agar for 28 days prior to their use in the experiments.

The results of these experiments are illustrated in Table I. It will be noted that all of the highest concentrations (5 per cent) of the various organ extracts in either liquid or solid medium caused an apparent cessation of growth of the virulent organisms. Kidney and heart extracts, producing complete inhibition of growth at 2 per cent, were apparently more effective than the other extracts. A marked difference between the response of the avirulent and virulent organisms to the various extracts was likewise apparent.

In order to determine whether the action of the organ extracts was bactericidal or bacteriostatic, the original inocula in all tubes of the H37 strain in which growth was suppressed were seeded upon glycerin-agar slants. Those organisms previously exposed to kidney, heart, brain, and liver extracts failed to grow, indicating bactericidal action for these extracts. The action of spleen extract, however, was apparently one of stasis, as organisms previously exposed to it grew profusely.

II.

Since the preceding experiments, showing inhibitory effects of tissue extracts on *M. tuberculosis*, had been conducted on a non-quantitative basis, a cultural technique was devised for a quantitative study of the effect of extracts of this nature. This technique, using a standard amount of inoculum, was an adaptation of the methods of Cooper and Cohn[7] and of Youmans[8, 9]. The surface-tension-reducing agent advocated

[7] Cooper, H. J., and Cohn, M. L.: *J. Lab. Clin. Med.* **21** (1935): 428.

[8] Youmans, G. P.: *Proc. Soc. Exptl. Biol. Med.* **57** (1944): 119.

[9] Youmans, G. P.: *Proc. Soc. Exptl. Biol. Med.* **57** (1944): 122.

TABLE II. *Quantitative Effects of Tissue Extracts on Growth of Tubercle Bacilli Using a Standard Inoculum.*

Organism	Avirulent ATCC No. 607		Virulent H37		Virulent G-2		Virulent G-5	
Extract Percent	Growth	Yield mg.	Growth	Yield mg.	Growth	Yield mg.	Growth	Yield mg.
Control	++++P*	17.7	++++P	4.5	++++P	3.4	++++P	2.1
			Brain					
0.5	++++P	24.2	++++P	5.5	+++	2.7	+++	1.5
1.0	−	−	+++P	4.2	++	1.8	+	0.2
2.0	−	−	+++P	3.6	+	1.1	+	0.1
5.0	−	−	+++	2.9	+	0.9	−	−
			Kidney					
0.5	++++P	46.7	++	1.3	+++	2.2	++	1.0
1.0	++++P	42.0	++	1.1	++	1.2	++	0.8
2.0	++++P	94.0	+	0.7	+	0.9	+	0.3
5.0	++++P	96.0	+	0.1	+	0.2	+	0.2
			Spleen					
0.5	++++P	86.0	++++P	4.8	++++P	3.3	++++P	2.1
1.0	+++	24.0	++++P	4.0	+++	2.3	++++P	1.8
2.0	−	−	++	2.3	+++	2.0	++	1.0
5.0	−	−	+	1.3	+	0.9	+	0.4
			Heart					
0.5	++++P	73.1	++++P	4.6	++++	2.8	++++P	2.3
1.0	++++P	58.1	++	1.8	++	1.1	++++P	2.0
2.0	−	−	+	0.2	+	0.3	+	0.5
5.0	−	−	−	−	+	0.1	+	0.1
			Liver					
0.5	++++P	68.0	+++P	3.3	+++	2.6	++++P	2.2
1.0	++++P	50.0	++	1.8	++	1.4	+++	1.1
2.0	++++P	36.0	++	1.2	++	1.1	+	0.5
5.0	++++P	30.6	+	0.7	+	0.5	+	0.2

*P Pellicle or surface growth; ++++ good growth; +++ growth; ++ slight growth; + very slight growth; − no growth.

by the former authors, and the emulsification procedure using an agate mortar and pestle, as described by Youmans, were omitted.

Ten milligrams of a 21-day-old culture of the organism grown on glycerin-agar slants were accurately weighed in a sterile test tube (Youmans). The culture was macerated for one minute by the stirring apparatus of Cooper and Cohn, and sterile medium was added. The mixture was thoroughly shaken before the addition of sufficient medium to give a final concentration of 1 mg. of organism per ml. of solution (Youmans). One-tenth milligram of the avirulent strain was seeded into control and experimental liquid-medium tubes prepared as in the non-quantitative experiments, and placed in the incubator at 37.5° C. for 7 days. One milligram of the virulent cultures was inoculated into a second series of tubes prepared in like manner and incubated for 21 days. All cultures were shaken after 3 and 5 days.

At the end of the incubation period, the weight of the organisms was obtained by a filtration method involving the use of alundum crucibles of fine porosity. The organisms were filtered in the weighed crucibles and boiling distilled water was poured through several times. The crucibles were then dried and re-weighed on the analytical balance. The results, presented in Table II, are similar in most respects to those in the non-quantitative investigations. It is of particular interest that kidney extract, which was one of the best inhibitors of growth for the virulent strains, was the best stimulator for growth of the avirulent organism.

DISCUSSION

Deproteinization and subsequent concentration increases the activity of substances in organ extracts which inhibit the growth of tubercle bacilli *in vitro*. The activity of the factor, which varies with the different organs, is greater in extracts of

the kidney, heart, and brain than in those obtained from the spleen and liver.

In accordance with the experience of Youmans[8, 9] we have also found that there is a difference in the response of infectious and non-infectious types of tubercle bacilli to various chemotherapeutic agents when tested *in vitro*.

Results of toxicity experiments are in process of preparation for publication. Animal experiments involving the use of tissue extracts are now in progress.

SUMMARY

1. A factor has been obtained from deproteinized beef kidney, heart, and brain extracts which, *in vitro*, shows germicidal and bacteriostatic activity in low concentrations (0.5-1.0 per cent) against virulent types of tubercle bacilli.

2. These extracts are unique in that they stimulate the growth of avirulent organisms at the same low level, but (with the exception of kidney extract) depress them at higher levels (2.0-5.0 per cent).

3. Spleen and liver extracts prepared in the same manner are somewhat less active against virulent strains of *M. tuberculosis* but react in much the same way to the avirulent forms.

4. Spleen extract at the 5 per cent level retards growth but all other tissue extracts tested show a bactericidal action at this level.

Original Manuscript Submitted
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